I. TOTAL SYNTHESIS OF (+)-SPONGIOLACTONE AND DERIVATIVES ENABLING INITIAL STRUCTURE-ACTIVITY RELATIONSHIP STUDIES: DISCOVERY OF A MORE POTENT DERIVATIVE

II. STUDIES TOWARD RECYCLABLE ISOTHIOUREA CATALYSTS

A Dissertation

by

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ABSTRACT

β-lactone containing natural products are attractive targets for total synthesis and subsequent biological studies. Given the remarkable ability of this moiety to covalently inhibit specific proteins, we were drawn to spongiolactone as a synthetic target.

Through our studies, the first total synthesis of this compound was achieved along with the synthesis of several derivatives. This synthesis enabled cytotoxicity studies to be carried out against a variety of cancer cell lines, with good activity observed in the human chronic myelogenous leukemia (K562) cell line. Particularly intriguing was the discovery of a more potent derivative than the natural product itself which was then modified and taken on to activity based protein profiling (ABPP) studies.

The first magnetite-supported benzotetramisole (BTM) catalyst was prepared and utilized in the kinetic resolution of secondary alcohols. Overall, the selectivity factor was lower than the parent (+)-BTM (s = 48 versus s = 148, respectively), however the resulting ester was achieved in excellent enantiomeric excess, albeit with low reaction conversion. Further studies suggest an unfavorable inactivation of the catalyst taking place in the presence of uncapped magnetite.

The first soluble, polymer-supported BTM catalyst was also synthesized using polyisobutylene as the support. Behavior of this catalyst system was superior to the magnetite-BTM adduct in the kinetic resolution of secondary alcohols and the conversion was efficient, providing the resolved alcohol and ester both in 90% *ee* which

corresponds to a selectivity factor of 79. Preliminary studies show problems of recyclability as indicated by the drop in selectivity factor (s = 51) and increase in reaction time to 16 hours. Further studies are necessary toward recoverability and recyclability of this system.

DEDICATION

To my parents, Steve and Debra Harvey

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I would like to thank my advisor, Prof. Daniel Romo, for his guidance during my graduate studies. The opportunity to complete a total synthesis allowed me to grow, not only in my abilities as a chemist and in problem-solving, but also as a person through the completion of a challenging project. I also am grateful for the liberty to pursue the project involving catalyst recyclability which was a personal interest and lastly, for the opportunity to complete an internship, which opened many doors and allowed me to begin my career.

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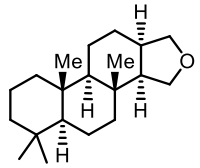
CHAPTER I

INTRODUCTION TO SPONGIANE DITERPENOID NATURAL PRODUCTS

1.1 Introduction

The enzyme-catalyzed cyclization of geranylgeranyl diphosphate leads to diverse structural skeletons broadly described as diterpenoids.[1] The biological value of these 20-carbon units has been proven with natural products such as taxol, driving forward the investigation into other diterpenoids. Following the isolation of several diterpenoids from marine sponges, all of which shared a common skeleton (**1.1**), the term spongiane diterpenoid was adopted in 1979 (Figure 1.1).



spongiane skeleton (**1.1**)

Figure 1.1 Common skeleton to spongiane diterpenoids.

1.2 Isolation of Spongiane Diterpenoids

The first spongiane diterpenoid, isoagatholactone (**1.2**), was isolated in 1974 by Minale and coworkers from the Mediterranean sponge *Spongia officinalis*.[2] (Figure 1.2).

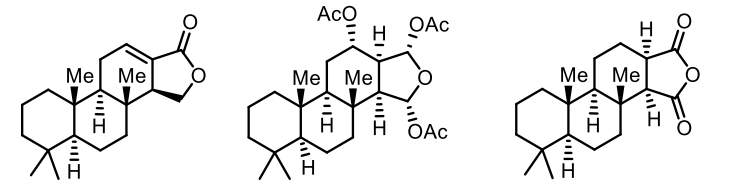


Figure 1.2 Examples of isolated spongiane diterpenoids.

Since this time, many other natural products have been added to this class of compounds, with varying levels of oxidation on the spongiane skeleton. Examples include aplysillin (**1.3)**, isolated off the coast of New Zealand from marine sponge *Aplysilla rosea*[3] as well as spongia-15, 16-dione (**1.4**)[4] from the sponge *Dictyodendrilla cavernosa*.

In 1985, the spongiane diterpenoid class of natural products was expanded beyond the originally defined tetracyclic core. During their studies of the metabolites of *Spongionella gracilis* isolated off the coast of Italy, Sica and coworkers discovered the first rearranged spongiane diterpenoid, norditerpene gracilin A (**1.5**) (Figure 1.3).[5]

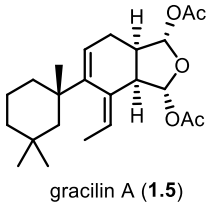


Figure 1.3 The first isolated rearranged spongiane diterpenoid.

Today, these rearranged natural products comprise the largest portion of known spongiane diterpenoids. Other closely related spongiane diterpenoids have been isolated, such as aplysillolide A (**1.7**) and B (**1.8**) from the sponge *Aplysilla glacialis* which contain the same core structure as gracilin A (**1.5**)[6] (Figure 1.4). The separately isolated natural product dendrillin (**1.9**) also contains a gracilin-type skeleton, albeit from Antarctic sponge *Dendrilla membranosa*.[7]

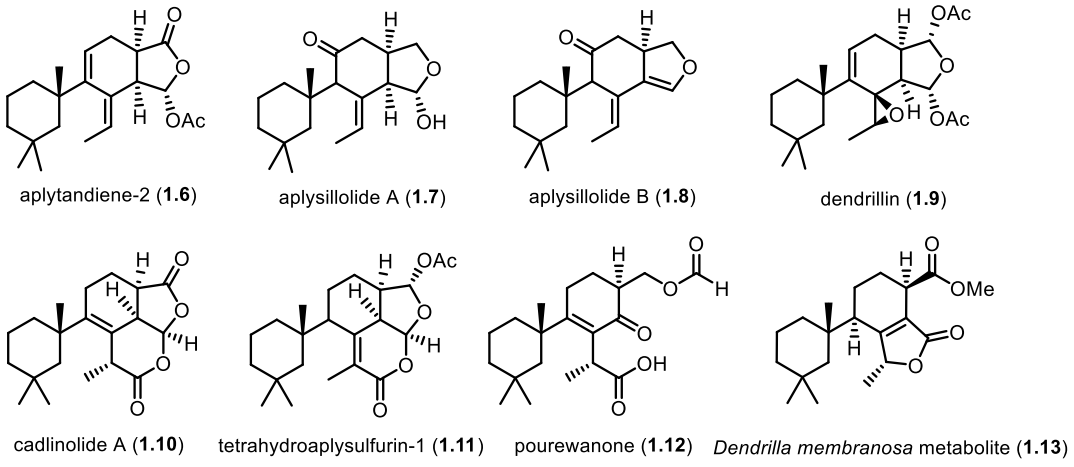
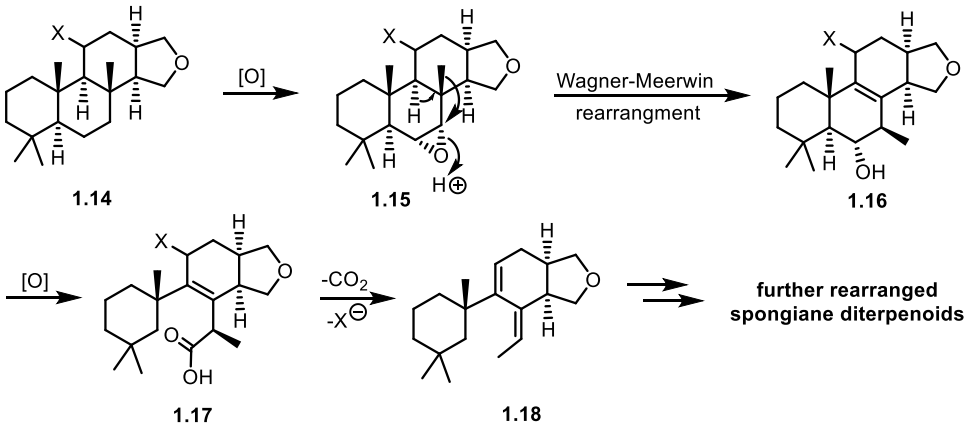


Figure 1.4 Other examples of rearranged spongiane diterpenoids.

Tricyclic diterpene lactones, such as cadlinolide A (**1.10**)[6] and tetrahydroaplysulfurin[6] (**1.11**) have demonstrated further structural diversity in the rearranged spongiane diterpenoids. Other isolates, such as pourewanone (**1.12**),[8] the first reported marine formate, and metabolites such as that from *Dendrilla membranosa* (**1.13**)[9] which contain a carboxylic acid or lactone, respectively.

The presence of these groups provides insight into a possible biosynthetic pathway (Scheme 1.1).

Scheme 1.1

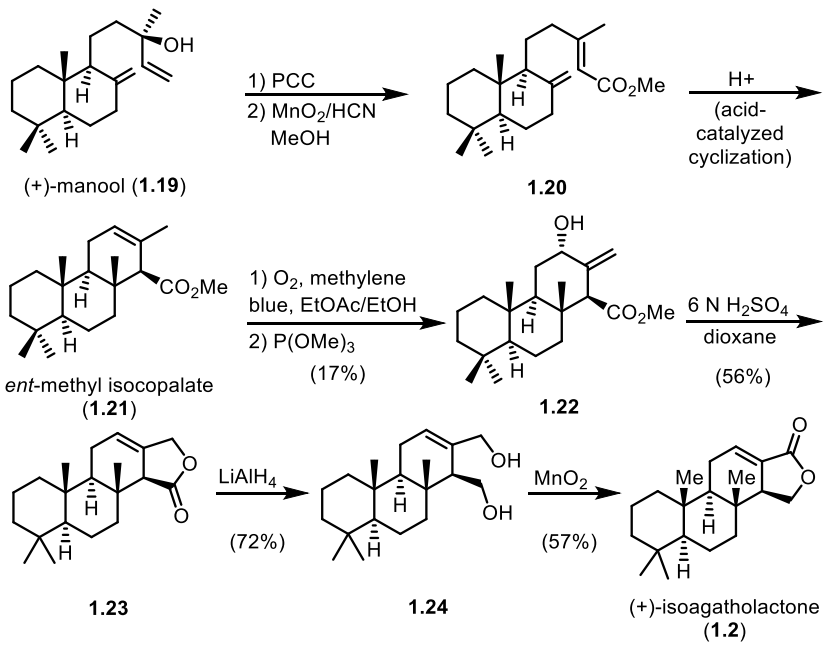


Here, it is thought that, following initial oxidation of the spongiane diterpenoid skeleton (**1.14**), a Wagner-Meerwein rearrangement takes place.[10] Further oxidation provides **1.17**, which, if followed by decarboxylation or cyclization, leads to the gracilins as well as tricyclic rearranged spongiane diterpenoids.

1.3 Selected Synthetic Studies of Spongiane Diterpenoids

The first reported synthesis of a spongiane diterpenoid was in 1981 by Rúrveda and coworkers, detailing the synthesis of (+)-isoagatholactone (**1.2**) (Scheme 1.2).[11]

Scheme 1.2

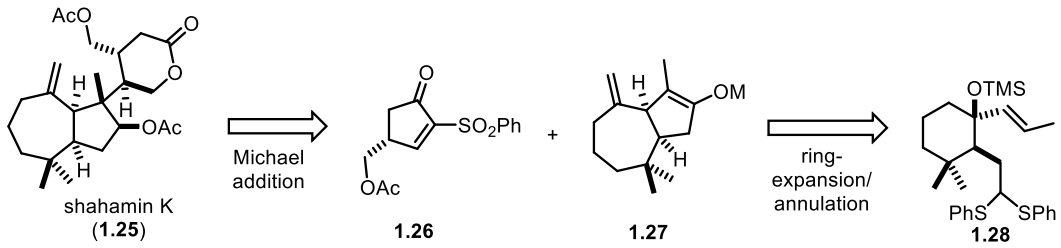


Beginning with oxidation of chiral starting material (+)-manool (**1.19**) followed by acid-catalyzed cyclization, known intermediate *ent*-methyl isocopalate (**1.21**) was formed. Subsequently, allylic alcohol (**1.22**) was accessed *via* photooxygenation which was followed by an allylic rearrangement/lactonization, providing **1.23**. Reduction of the lactone and oxidation/lactonization provided (+)-isoagatholactone (**1.2**) for the first time.

The first reported total synthesis of a rearranged spongiane diterpenoid was by Overman and coworkers in 2001 where they were able to not only access (+)-shahamin K (**1.25**) in a laboratory setting for the first time, but also confirm its relative and absolute stereochemistry.[12]

Retrosynthetically, it was envisioned that (+)-shahamin K could ultimately be derived from a Michael addition between the enolate of hydroazulene **1.27** and the corresponding cyclopentenone (**1.26**) (Scheme 1.3). Hydroazulene **1.27** would be constructed via a key ring expansion/annulation of the elaborated cyclohexane precursor **1.28**.

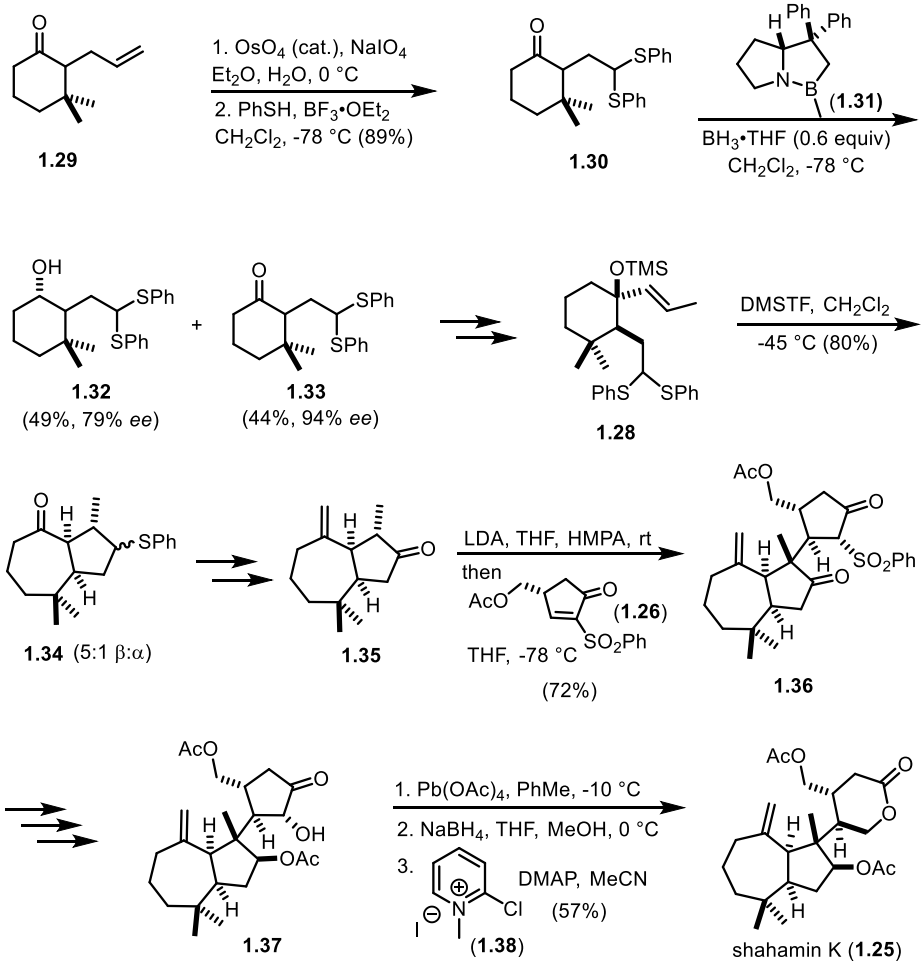
Scheme 1.3



Beginning with cyclohexanone **1.29**, Lemieux–Johnson oxidation of the olefin followed by dithioacetal formation afforded **1.30** which was then subjected to kinetic resolution to achieve highly enantioenriched **1.33** (Scheme 1.4). Subsequent transformations led to silyl ether **1.28** that underwent a Prins-pinacol rearrangement mediated by dimethyl(methylthio)sulfonium tetrafluoroborate (DMTSF) to give *cis* hydroazulene **1.34**. Upon enolate formation of cyclopentanone **1.35**, Michael addition to **1.26** successfully formed the key C8-C14 bond. Additional manipulations allowed them to arrive at advanced intermediate **1.36** which upon oxidation mediated by lead

tetraacetate, reduction of the resulting aldehyde, and lactonization afforded (+) shahamin K (**1.25**).

Scheme 1.4

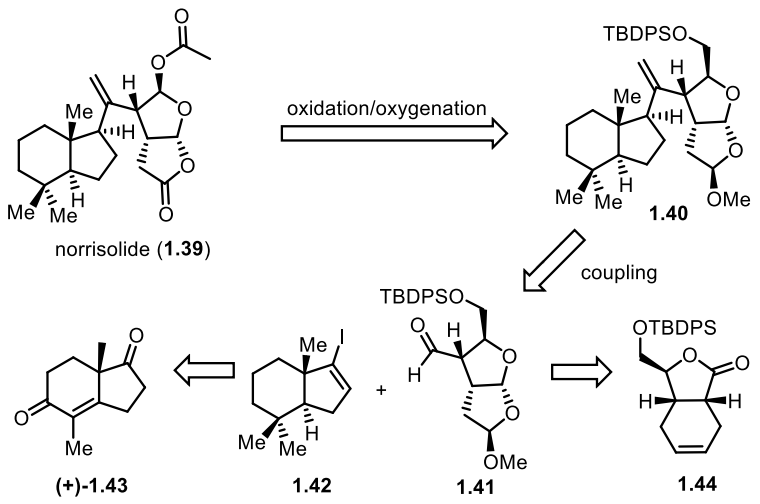


Adding to the reported chemistries of the spongiane diterpenoid family, the enantioselective synthesis of (+)-norrisolide (**1.39**) was reported in 2004 by Theodorakis and coworkers.[13] From the structural standpoint, norrisolide presented an interesting

challenge as it contains a γ-lactone, γ-lactol ring system. Norrisolide was the first spongiane diterpenoid with this highly oxygenated moiety to succumb to total synthesis.

Retrosynthetically, it was thought that late stage oxygenation of a highly advanced intermediate (**1.40**) would provide access to norrisolide (Scheme 1.5). This late stage intermediate could arise from a direct coupling between vinyl iodide **1.42** and aldehyde **1.41**, where **1.42** could be derived from enone **(+)-1.43** and aldehyde **1.41** could come from lactone **1.44**.

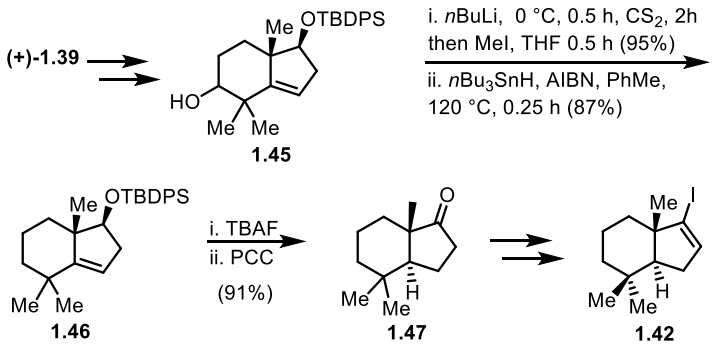
Scheme 1.5



The forward route began with optically active enone **(+)-1.39** which was converted to silyl ether **1.45** over a few steps (Scheme 1.6). This was followed by Barton-McCombie radical deoxygenation to access bicyclic cyclopentene **1.46** in

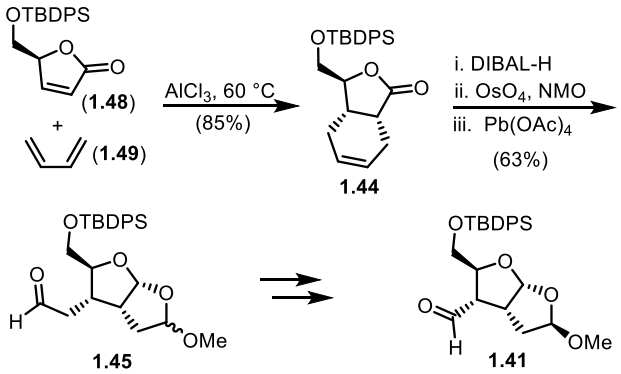
excellent yield. Subsequent desilylation and oxidation afforded cyclopentanone **1.47** which was taken on in a few steps to the desired vinyl iodide **1.42**.

Scheme 1.6



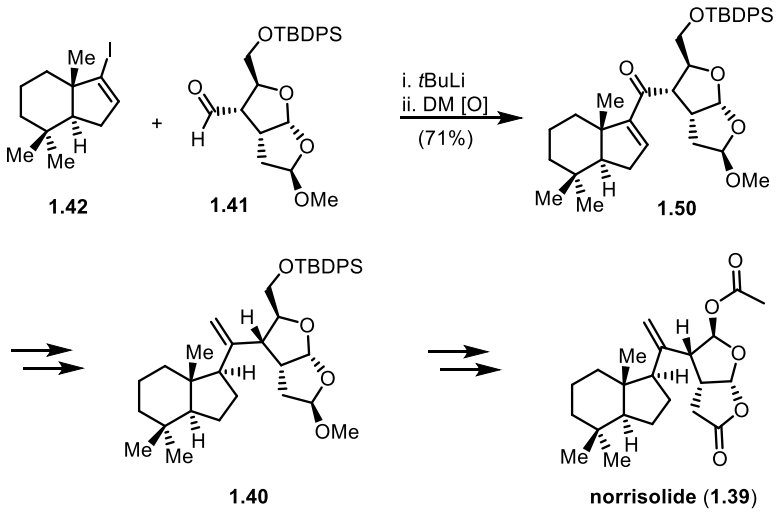
Accessing aldehyde coupling partner **1.41** began with a Diels-Alder reaction to afford bicyclic cyclohexene **1.44** as a single isomer with the facial preference controlled by the bulky silyl group (Scheme 1.7). DIBAL-H reduction followed by dihydroxylation and oxidation yielded requisite aldehyde **1.45** which was taken on in a few steps to achieve necessary coupling partner **1.41**.

Scheme 1.7



With the two pieces in hand, lithium halogen exchange of **1.42** afforded the appropriate nucleophile for aldehyde **1.41** and the resulting aldol adduct was oxidized to give ketone **1.50** that was further elaborated to **1.40** and, ultimately, norrisolide **1.39** (Scheme 1.8).

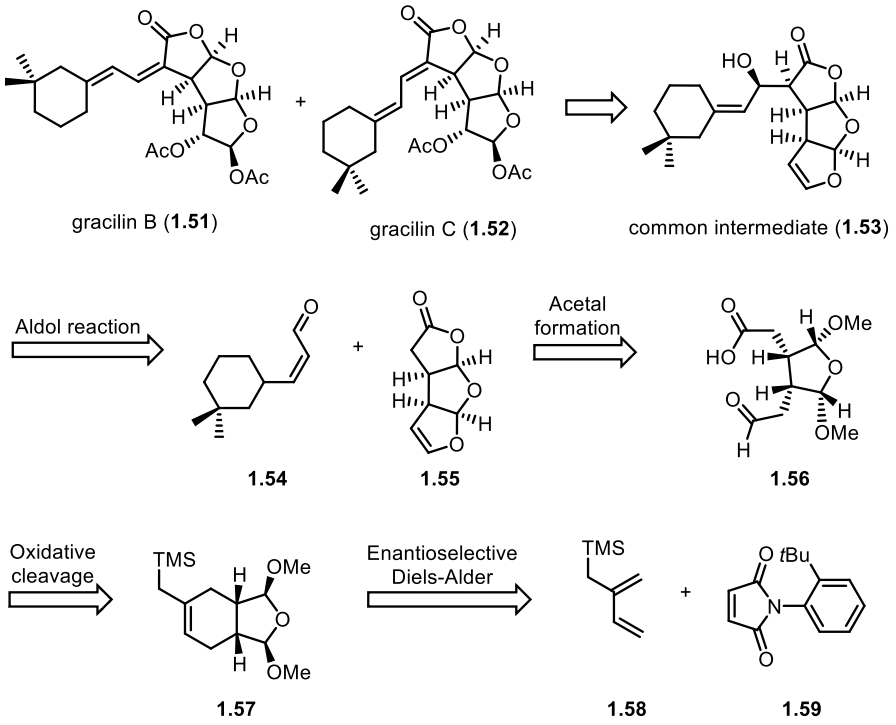
Scheme 1.8



The first total synthesis of members of the gracilin family of rearranged spongiane diterpenoids was reported in 1995 by Corey and coworkers.[14] Given their previously published catalytic, highly enantioselective Diels-Alder reaction,[15] they not only sought to access this family of natural products for the first time but also saw these targets as a powerful synthetic application for their methodology.

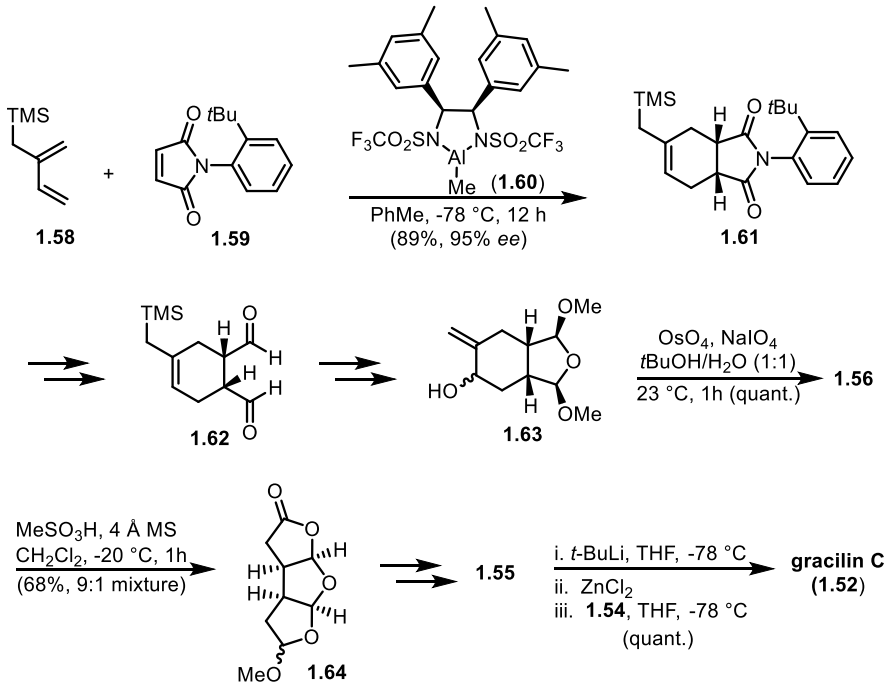
Retrosynthetically, it was envisioned that both gracilin B (**1.51**) and gracilin C (**1.52**) could both be accessed from late-stage alcohol **1.53** (Scheme 1.9). This common intermediate could be formed via an aldol reaction between aldehyde **1.54** and tricyclic lactone **1.55**. Lactone **1.55** could arise through acetal formation of an aldehyde acid precursor **1.56** that could be formed through oxidative cleavage of bicyclic cyclohexene acetal **1.57** that would ultimately be achieved from an enantioselective Diels-Alder reaction between diene **1.58** and dienophile **1.59**.

Scheme 1.9



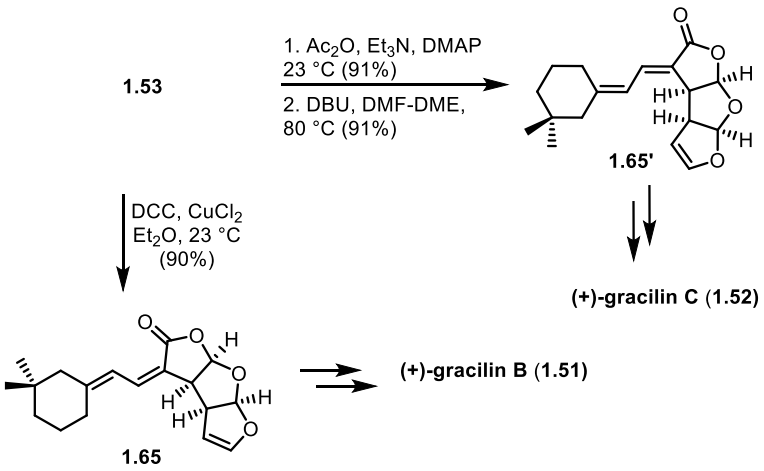
The synthesis commenced with the previously developed enantioselective Diels-Alder reaction to provide cyclohexene **1.61** in excellent yield and *enantiomeric excess* (Scheme 1.10). Subsequent steps provided dialdehyde **1.62** which was advanced to cyclic *bis* acetal **1.63**. Oxidative cleavage mediated by osmium tetroxide provided aldehyde acid **1.56** which formed tricyclic lactol **1.64** under acidic conditions. This lactol was converted to tricyclic vinyl ether lactone **1.55** and a final aldol reaction with aldehyde **1.54** gave common intermediate **1.53**.

Scheme 1.10



Access to both gracilin B and C was achieved by intermediate **1.53** (Scheme 1.11). For gracilin C, acetylation of the secondary alcohol followed by elimination afforded triene **1.65'** in excellent yield which was easily transformed into gracilin C (**1.52**) through epoxidation, acetolysis, and acetylation. Accessing gracilin B utilized stereospecific dehydration to provide the necessary triene (**1.65**) which was taken through the same sequence to achieve gracilin B (**1.51**).

Scheme 1.11



1.4 Biological Studies of the Spongiane Diterpenoids

The spongiane diterpenoids display a broad spectrum of biological activity including, but not limited to anticancer, inhibition against tyrosine kinase, phospholipase A_2 (PLA_2)/anti-inflammatory, and neoroprotective properties. Extensive investigation into gracilins B (**1.51**) and C (**1.52**) (Figure 1.5) showed micromolar GI_{50} values (concentration of compound of interest required to cause 50% growth inhibition) in twelve different cancer cell lines including pancreatic, cervical, prostate, lung, breast, ovarian, colon, leukemia, and melanoma.[16,17]

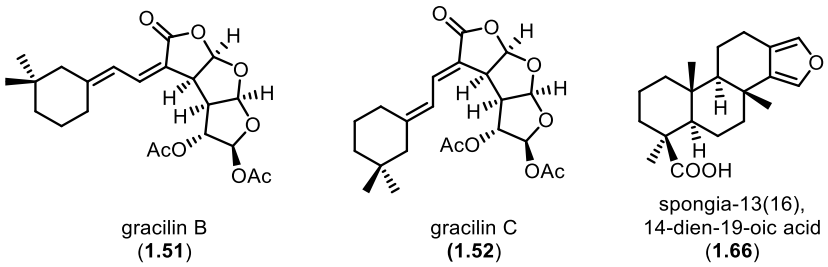


Figure 1.5 Spongiane diterpenoids that display anticancer activity.

Toward specific treatment of prostate cancer, spongia-13(16), 14-dien-19-oic acid **1.66** was found to block androgen receptor transcription and specifically inhibit cell-proliferation that relies on androgen.[18]

Gracilin A (**1.5**) has demonstrated up to 69% inactivation of phospholipase A_2, which has implications toward anti-inflammatory activity. A separate investigation (Figure 1.6) of cadlinolide C (**1.67**), methylpourewate B (**1.68**),and pourewic acid A (**1.69**) also showed moderate levels of anti-inflammatory activity.[8]

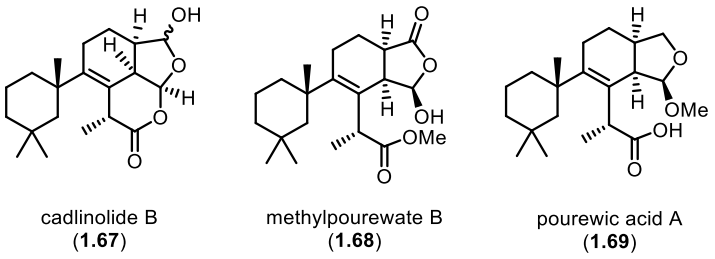


Figure 1.6 Spongiane diterpenoids displaying anti-inflammatory activity.

More recent literature reported spongiane diterpenoids, specifically tetrahydroaplysulphurin-1 (**1.11**) along with gracilins A (**1.5**), H (**1.70**), K (**1.72**), J (**1.71**), and L (**1.73**) (Figure 1.7) to have neuroprotective properties.[19]

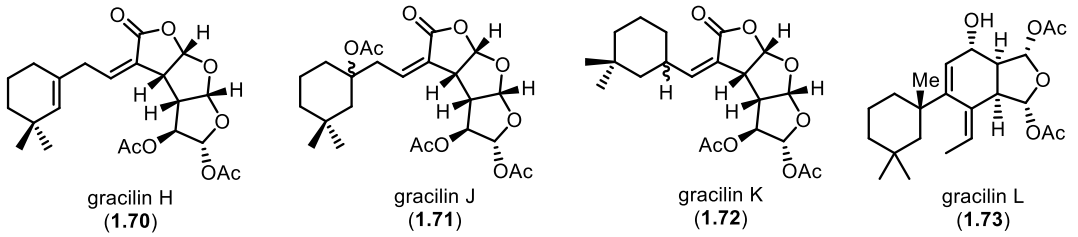


Figure 1.7 Members of the gracilin family that display neuroprotective properties.

In these studies, the compounds mentioned were used in the presence of hydrogen peroxide, which is known to induce oxidative stress, and the level of oxidative damage on the cells was measured. Tetrahydroaplysulphurin-1 (**1.11**) showed exceptional results, completely protecting against neurodamage.

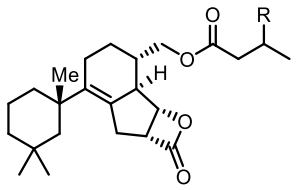
Gracilins A (**1.5**), H (**1.69**), and L (**1.72**) along with tetrahydroaplysulphurin-1 **2.x** were also evaluated for their ability to reduce hyperphosphorylation of tau proteins, which is linked to the progression of Alzheimer's disease.[20] Gracilins H (**1.70**) and L (**1.73**) were even evaluated *in vivo* in chronic Alzheimer's disease mice which showed, after treatment, improved spatial memory and learning.

CHAPTER II

TOTAL SYNTHESIS OF (+)-SPONGIOLACTONE AND RELATED COMPOUNDS*

2.1 Introduction

Presenting a highly rearranged spongiane skeleton and a unique fused β-lactone, (+)-spongiolactone (**2.1**) was first isolated in 1986 by Sica and coworkers from the sponge *Spongionella gracilis* (Figure 2.1).[21]



(+)-spongiolactone (**2.1**, R = Me)
(+)-C3'-norspongiolactone (**2.2**,R = H)

Figure 2.1 β-lactone containing spongiane diterpenoids.

In 2009, a related congener was isolated by Jaspers, C3'-norspongiolactone (**2.2**), differing only by a methyl group in the side-chain ester, and found to possess antiproliferative activity toward human chronic mylogenous leukemia cells (K562, IC_{50} 12 ± 1 μM) and potential immunosuppressive activity through inhibition of human peripheral blood mononuclear cells (PBMC, IC_{50} 30 ± 10 μM).[17] Despite the presence of

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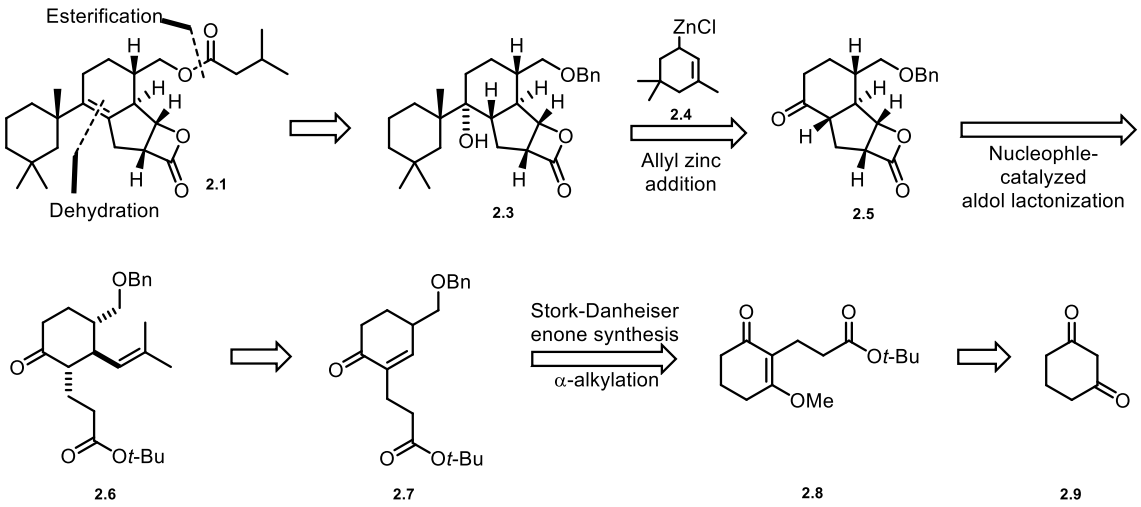
* Reprinted with permission from "Synthesis of (±)-Spongiolactone Enabling Discovery of a More Potent Derivative" by Harvey, N. L.; Krysiak, J.; Chamni, S.; Cho, S. W.; Sieber, S. A.; Romo, D. *Chem. Eur, J.* **2015**, *21*, 1425-1428. Copyright 2015 John Wiley and Sons, Weinheim. See Appendix C.

the resident β-lactone, with potential as a protein-reactive, acylating pharmacophore, neither synthetic nor mechanism of action studies have been reported for the spongiolactones.

2.1.1 Previous Studies Toward Spongiolactone

The initial retrosynthetic analysis envisioned accessing (+)-spongiolactone by a late stage esterification and dehydration of elaborated tricyclic β-lactone **2.1** (Scheme 2.1). The all-carbon quaternary center directly adjacent to the tertiary alcohol of **2.3** would be installed by addition of separately prepared allyl zinc reagent **2.4** to tricyclic β-lactone **2.8** which could arise via a nucleophile-catalyzed aldol lactonization of an intermediate aldehyde-acid derived from trisubstituted cyclohexanone **2.6**. The cyclohexanone could come from a cuprate addition to enone **2.7** that could easily be derived utilizing a Stork-Danheiser enone synthesis of β-keto enol ether **2.8** which could be formed from commercially available 1,3-cyclohexanedione **2.9**.

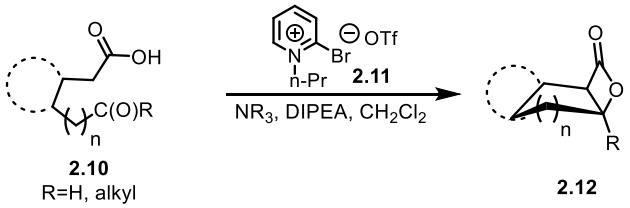
Scheme 2.1



2.1.2 Synthesis of β-lactones via the Intramolecular, Nucleophile Catalyzed Aldol-Lactonization (NCAL) Process

In the Romo group, extensive work has been contributed to the literature detailing the synthesis of β-lactones. Specifically, this work has focused on a nucleophile catalyzed aldol-lactonization process developed in the group. Since its seminal report[22], this process has evolved with respect to the substrate scope amenable to the transformation from aldehyde acids[23-26] to keto acids[27, 28] (Scheme 2.2).

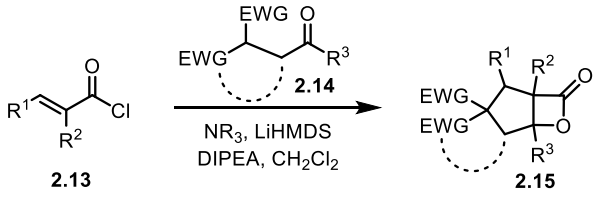
Scheme 2.2



This methodology has been carried out in a double diastereoselective fashion with optically active aldehyde acids[25] with an extension to β-lactone fused tetrahydrofurans, and further work displayed the use of keto acids to generate bi- and tri-cyclic β-lactones in an enantioselective, organocatalytic NCAL process.[28]

Most recently, a nucleophile catalyzed, Michael aldol-β-lactonization (NCMAL) organocascade[29] (Scheme 2.3) was described to rapidly generate complex cyclopentanes (**2.15**).

Scheme 2.3



Utilizing commodity acid chlorides (**2.13**), a highly reactive, chiral α, β-unsaturated acylammonium intermediate is generated which then participates in the

organocascade process to generate up to three contiguous stereogenic centers, two rings, one C-O bond, and two C-C bonds in high enantiomeric excess.

The β-lactones synthesized by these methodologies have been used to access β-lactone containing natural products, such as (-)-salinosporamide A **2.15**, as well as to set key stereogenic centers in a molecule[30-32] through further manipulations: a strategy used toward (+)-dihydroplakevulin A (**2.16**),[27] (-)-curcumanolide A (**2.17**),[32] and (+)-omphadiol (**2.18**) (Figure 2.2).[31]

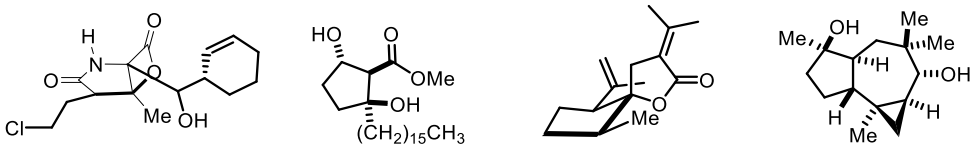
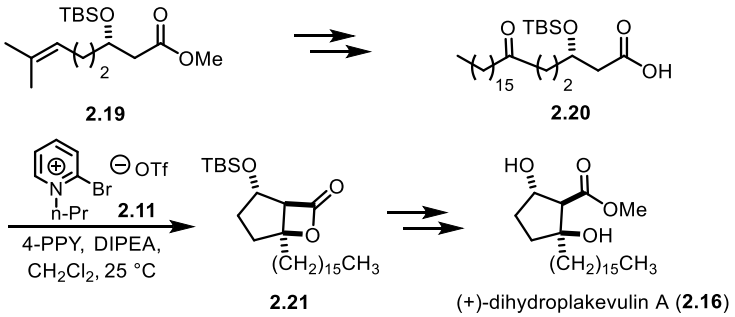


Figure 2.2 Natural products synthesized with the NCAL as a key step.

The total synthesis of (+)-dihydroplakevulin **2.16** was the fruit of the extension of the NCAL process to keto acid substrates (Scheme 2.4).[27] Before these reported studies, the yields for these substrates were <5%.

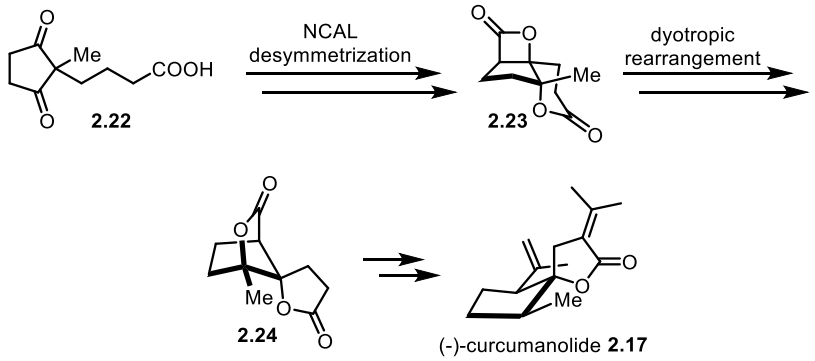
Scheme 2.4



Ultimately, switching to pyridine derivatives, such as 4-pyrrolidinopyridine, which have a higher nucleophilicity than the previously examined triethylamine, enabled the bis-cyclization process to proceed and opened the door to asymmetric transformations. With the required stereogenic centers of the molecule set by the β-lactonization process, (+)-dihydroplakevulin was accessed by a few further functional group interconversions.

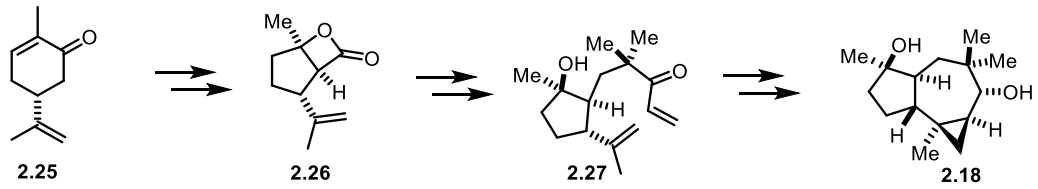
To access (-)-curcumanolide A (**2.17**), the NCAL reaction was applied to keto acid **2.22** to form tricyclic β-lactone **2.23** (Scheme 2.5). This β-lactone then underwent a dyotropic rearrangement to provide spiro-γ-butyrolactone **2.24** which was then taken on to the natural product.

Scheme 2.5



A third example of using an intermediate β-lactone to set key stereogenic centers in a total synthesis is (+)-omphadiol **2.18** (Scheme 2.6). In this case, a keto acid derived from (*R*)-carvone **2.25** was subjected to the NCAL process, leading to β-lactone **2.26** in highly diastereoselective fashion. This key intermediate was further manipulated to access (+)-omphadiol **2.18** for the first time.

Scheme 2.6

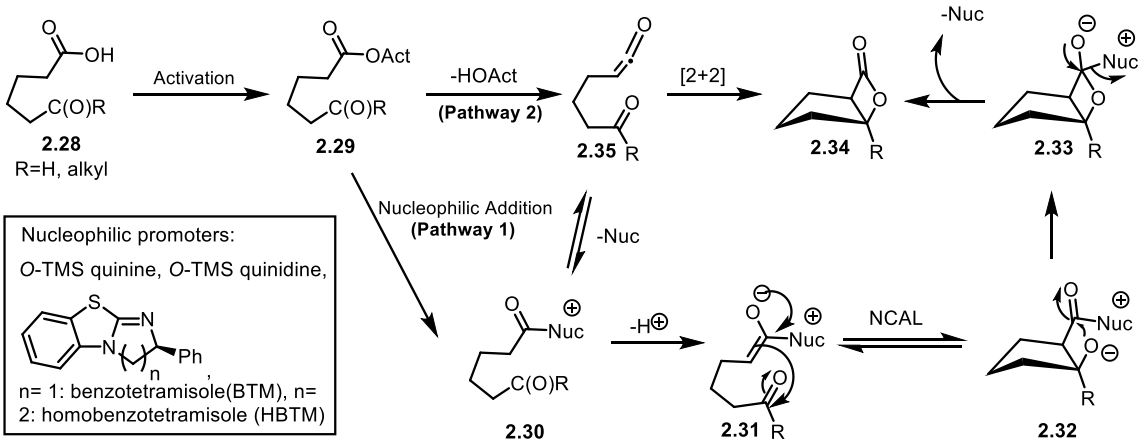


2.1.3 Proposed Mechanism for the Intramolecular, Nucleophile Catalyzed Aldol-Lactonization (NCAL) Process

Mechanistically, the nucleophile catalyzed aldol-lactonization process begins by activation of the carboxylic acid of the parent aldehyde acid or keto acid **2.28** (Scheme 2.7). Activated intermediate **2.29** can then undergo two separate pathways. Following Pathway 1, nucleophilic addition by a tertiary amine, such as *O*-TMS quinine or *O*-TMS quinidine, or an isothiourea catalyst, such as benzotetramisole (BTM) or homobenzotetramisole (HBTM), leads to acylammonium **2.30**. Deprotonation of acylammonium **2.30** leads to ammonium enolate intermediate **2.31** that can then undergo an aldol reaction with the pendant carbonyl leading to **2.32**. This aldol adduct can then undergo β-lactonization and turnover the catalyst.

Alternatively, activated intermediate **2.29** can form ketene **2.35** via Pathway 2. It should be noted that the same ketene can result from acylammonium **2.30**. This ketene can then undergo a [2 + 2] cycloaddition with the pendant carbonyl yielding the same β-lactone **2.34** as Pathway 1.

Scheme 2.7



From the studies carried out in the Romo group, Pathway 1 is favored given the ability to generate β-lactones in high enantiomeric excess, indicating the chiral nucleophilic promoter is present during the aldol process.

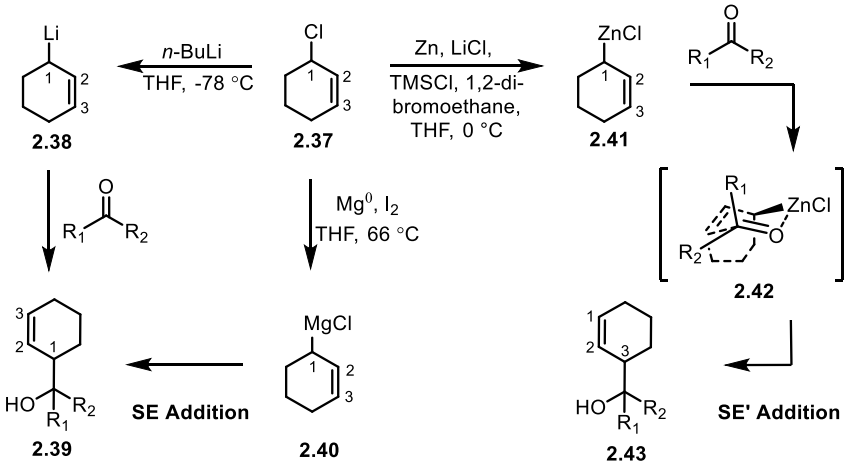
2.1.4 Allylzinc Reagents as a Means to Install All-Carbon Quaternary Centers

The controlled formation of all-carbon quaternary centers continues to challenge synthetic chemists. Several methods are available to complete this task,[33-35] including but not limited to alkylations, conjugate additions, rearrangements, Diels-Alder reactions, and allylic substitutions. In considering the system specific to spongiolactone, we were drawn to allyl zinc reagents as a means to forge a necessary all-carbon quaternary center adjacent to a tertiary alcohol.

Allyl zinc reagents are known to undergo S_E' addition to carbonyls in a highly regioselective manner, predicated on a proposed highly ordered chair-like, closed

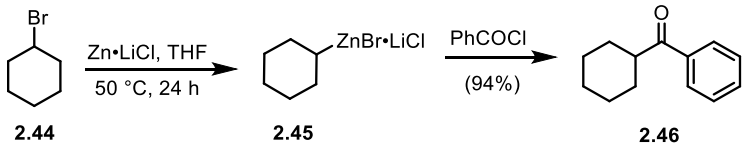
transition state arrangement **2.42** (Scheme 2.8),[36] are tolerant to other functional groups, and are relatively stable. This is in contrast to allylic magnesium and lithium species which are highly reactive and often unstable.

Scheme 2.8



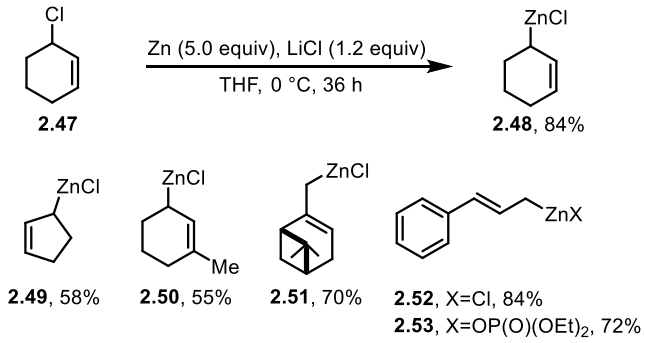
The preparation of allyl zinc reagents was not always a straightforward process. In early studies toward allyl zinc reagents, the use of highly reactive zinc, such as Rieke zinc,[37] made their use less appealing. In 2006, Knochel and coworkers reported milder conditions[38] utilizing lithium chloride to facilitate the formation of alkyl and aryl-zinc compounds with commercially available zinc dust (Scheme 2.9).

Scheme 2.9



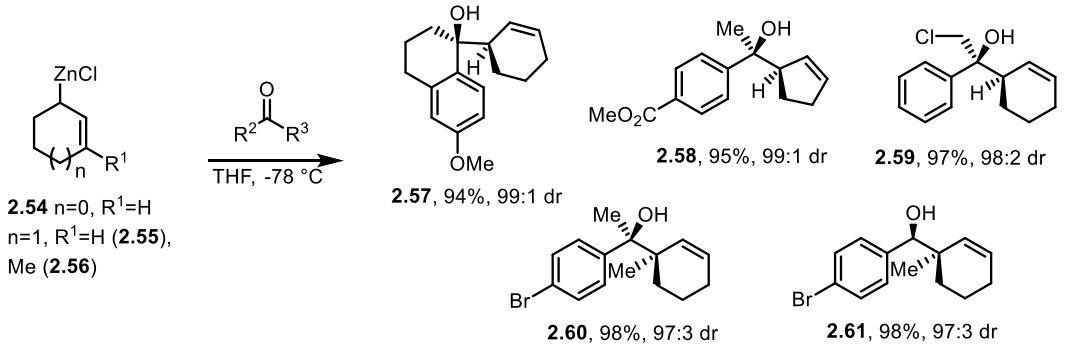
This work was centered around the use of either iodine or bromine as the halogenated precursor (**2.44**), since these species are more reactive toward formation of the zinc reagent. Specifically considering substituted alkyl systems, this high reactivity was detrimental and, in fact, large amounts of homocoupling was observed.[36] In a follow-up report in 2007, Knochel and coworkers detailed the use of less reactive substituted allyl chlorides (**2.47**) in the presence of lithium chloride, under mild reaction conditions, to overcome this problem (Scheme 2.10).

Scheme 2.10



The prepared allyl zinc reagents were added to a variety of carbonyl electrophiles to produce all-carbon quaternary centers adjacent to tertiary alcohols in excellent yield and diastereoselectivity (Scheme 2.11).

Scheme 2.11



2.54 n=0, R^1=H
n=1, R^1=H (**2.55**),
Me (**2.56**)

2.57, 94%, 99:1 dr

2.58, 95%, 99:1 dr

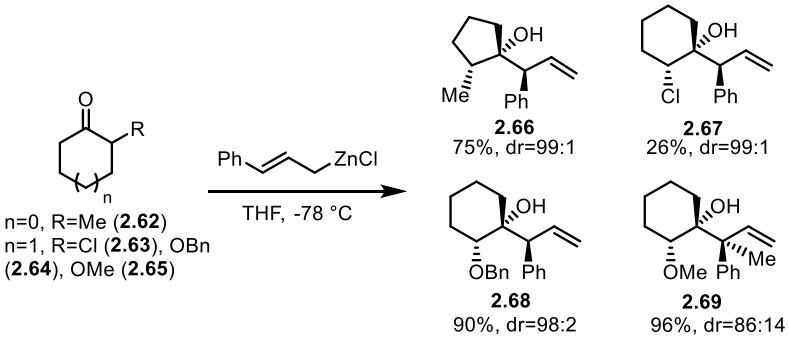
2.59, 97%, 98:2 dr

2.60, 98%, 97:3 dr

2.61, 98%, 97:3 dr

Applying these reagents to more complex systems, studies were reported on α-chiral carbonyl compounds (Scheme 2.12). In this case, a variety of α-substituents (**2.62-2.65**) were examined, all providing excellent diastereoselectivity and yield, analogous to previous studies on simpler systems.

Scheme 2.12



While these results show the utility of allyl zinc reagents to install all-carbon quaternary centers, the most substituted systems for which this reaction is applied contained only an α-substituent.

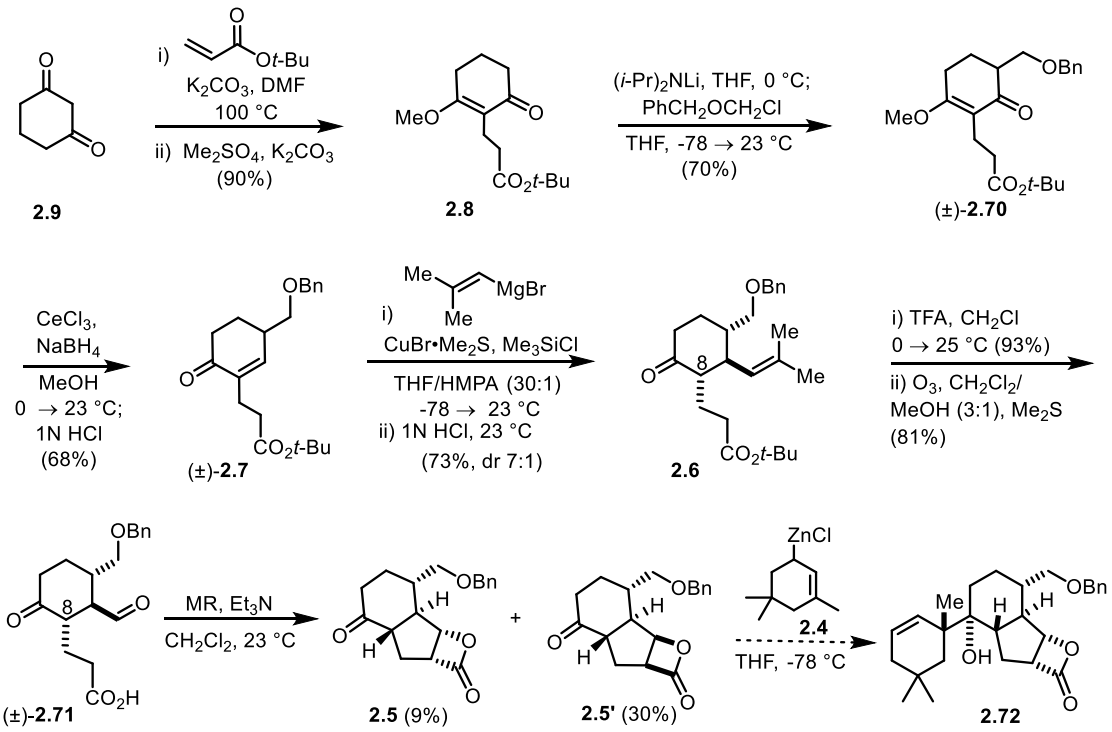
2.2 Results and Discussion

2.2.1 Synthesis of Tricyclic β-lactone Core Toward Spongiolactone and Attempted S_E' Addition

The synthesis commenced with a scaleable two-stage, one-pot sequence involving conversion of 1,3-cyclohexanedione (**2.9**) to β-keto enol ether **2.8** through an initial Michael addition with *t*-butyl acrylate followed by *O*-alkylation in 90% overall yield (Scheme 2.13). Application of a kinetic alkylation with benzyl chloromethyl ether exclusively afforded α-benzyloxymethylene adduct (±)-**2.70** through careful control of reaction temperature to ensure kinetic deprotonation. A Stork-Danheiser cyclohexenone synthesis[39] involving Luche reduction[40] and acidic workup directly provided

cyclohexenone (±)-**2.7**. Addition of isobutenyl cuprate to this enone in the presence of Me_3SiCl provided a single diastereomer (>19:1 by 500 MHz ^1H NMR) of an intermediate silylenol ether[41] which was typically directly desilylated through an acidic workup to yield the *anti*,*anti*-trisubstituted cyclohexanone (±)-**2.6** as an inseparable 7:1 mixture of diastereomers, epimeric at the C8-stereocenter.

Scheme 2.13

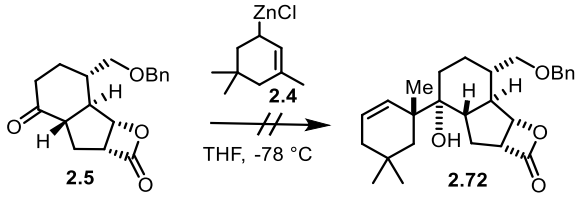


Treatment with trifluoroacetic acid (TFA) easily converted the *t*-butyl ester to the carboxylic acid. Ozonolysis of the trisubstituted olefin afforded aldehyde acid (±)-**2.71**

which was then applied to the NCAL process to give products **2.5** and **2.5'** which are diastereomeric at the β-lactone.

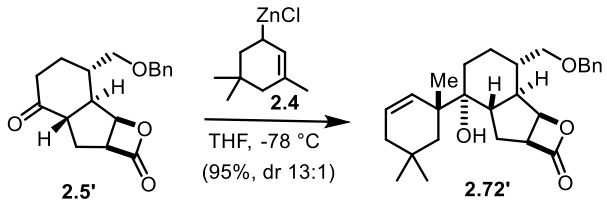
With β-lactone **2.5** in hand, the next step was the chemoselective S_E' addition of allyl zinc reagent **2.4** to ketone **2.5**. To our disappointment, the desired transformation did not occur (Scheme 2.14) and the starting material was recovered even if the reaction was warmed to 0 ˚C. It should be noted that another general problem with this reaction was the inconsistent preparation of allyl zinc reagent **2.4**.

Scheme 2.14



Interestingly, when the same reaction conditions were applied to diastereomeric β-lactone **2.5'**, the reaction proceeded in excellent yield and diastereoselectivity to give **2.72'** (Scheme 2.15). Having reached an impasse, the synthetic approach was reevaluated to try to circumvent this problematic step.

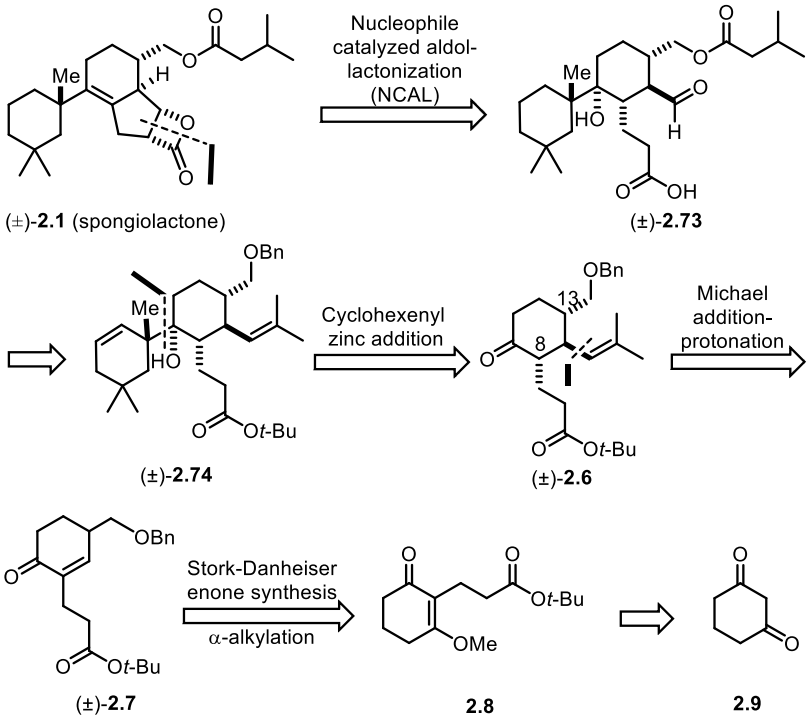
Scheme 2.15



2.2.2 Modified Retrosynthetic Analysis of (±)-Spongiolactone

The modified retrosynthetic analysis toward spongiolactone invoked a late-stage NCAL process with racemic aldehyde acid (±)-**2.73** (Scheme 2.16). The required aldehyde acid (±)-**2.73** would be secured through standard functional group manipulations following a challenging S_E' cyclohexenyl zinc addition to α-substituted ketone (±)-**2.6** with the stereochemical outcome predicated on a chair-like, closed transition state arrangement[36, 42] guided by the cyclohexyl C8-substituent (spongiolactone numbering). The tri-substituted, all *anti*-cyclohexanone (±)-**2.6** would be accessed through a Michael addition expected to occur *anti* to the existing γ-benzyloxymethylene C13-substituent and subsequent protonation/equilibration at C8. α-Alkylation of enol ether **2.8**, derived from 1,3-cyclohexanedione, with benzyloxymethyl chloride followed by application of a Stork-Danheiser enone synthesis would deliver cyclohexenone (±)-**2.7**.

Scheme 2.16

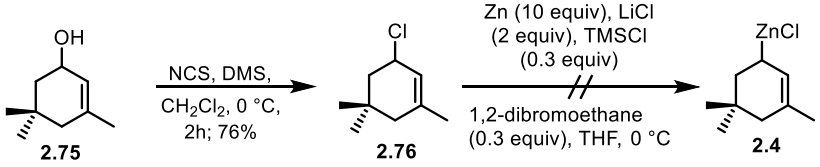


2.2.3 Development of Consistent Cyclohexenylzinc Reagent Preparation

With the desired trisubstituted cyclohexanone (±)-**2.6** already in hand, we next studied the challenging S_E' addition of cyclohexenyl zinc chloride **2.4** that would install two adjacent quaternary carbons including one all-carbon stereocenter. Initially, efforts to consistently form the desired cyclohexenylzinc reagent followed closely to that reported by Knochel and coworkers (Table 2.1).[36] Though the allyl chloride required in our efforts toward spongiolactone had not been reported previously, conversion to allyl chloride **2.76** from commercially available cyclohexenol **2.75** proceeded in good yield

and was used without further purification. Problems were encountered, however, in the conversion from allyl chloride **2.76** to allyl zinc reagent **2.4**. Either the allyl zinc reagent would be completely inactive, as determined by titration with iodine as described previously,[36] or the concentrations were <0.01 M and quickly diminished after only a few hours (Table 2.1, Entry 1).

Table 2.1 Optimization of allyl zinc reagent preparation.



entry	modification to conditions	Result
1	-	<0.02 M
2	Allyl chloride 2.x purified	Inactive
3	Allyl chloride 2.x freshly prepared	0.05 M after 15 h
4	Flame-dried zinc	Visible activation with only addition of 1,2-dibromoethane, TMSCl added, inactive
5	**TMSCl excluded**	**0.06 M, reagent 2.4 stable up to 1 week at -20 ˚C**

Initial efforts to overcome these problems focused on allyl chloride **2.76**. Since this intermediate is unstable, purification was initially avoided and it was reasoned that impurities may be causing problems with the allyl zinc formation, however the results did not change when purified **2.76** was used (Entry 2). Freshly preparing **2.76** (Entry 3)

did ultimately yield active allyl zinc reagent **2.4** though the reaction was sluggish and not reproducible over several trials.

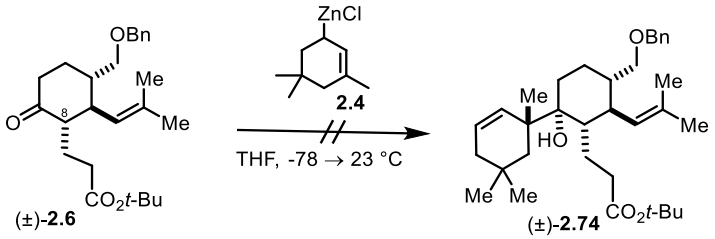
Since experimenting with **2.76** did not lead to improvements in preparing the allyl zinc reagent, the focus shifted to the zinc itself. Attempted activation of Zn^0 powder with HCl led to no improvement in the reaction. Flame drying both Zn^0 and LiCl showed promise. Specifically, it was noted that when the zinc was flame dried (Entry 4), activation, indicated by vigorous bubbling, proceeded with only the addition of 1,2-dibromoethane, though adding trimethylsilyl chloride (TMSCl) as carried out in the literature resulted in inactive reagent. This led us to question whether the TMSCl was truly necessary to effect the desired transformation. Pleasingly, it was found that exclusion of this reagent resulted in consistent preparation of **2.4** (Entry 5) and, further, that this reagent was stable for up to a week when stored under nitrogen at low temperatures.

The addition of LiCl was previously shown by Knochel to facilitate zincate formation.[38, 43] Addition of Me_3SiCl has also commonly been employed to assist with zincate formation,[44] however the exclusion of this additive ultimately enabled consistent concentrations (0.2-0.5 M in THF) of the targeted (±)-**2.4** to be prepared. However, numerous attempts to add (±)-**2.4** to ketone (±)-**2.6** under a variety of reaction conditions were unsuccessful (Table 2.2).

It was found that changing the number of equivalents of allyl zinc reagent had no effect on the outcome of the reaction (Table 2.2). Likewise, varying the overall reaction concentration failed to forge the S_E' addition. Only upon heating the reaction mixture

was any addition observed, though the chemoselectivity was lost with S_E addition taking place at the *t*-butyl ester (Entry 6).

Table 2.2 Reaction conditions screened toward S_E' addition.

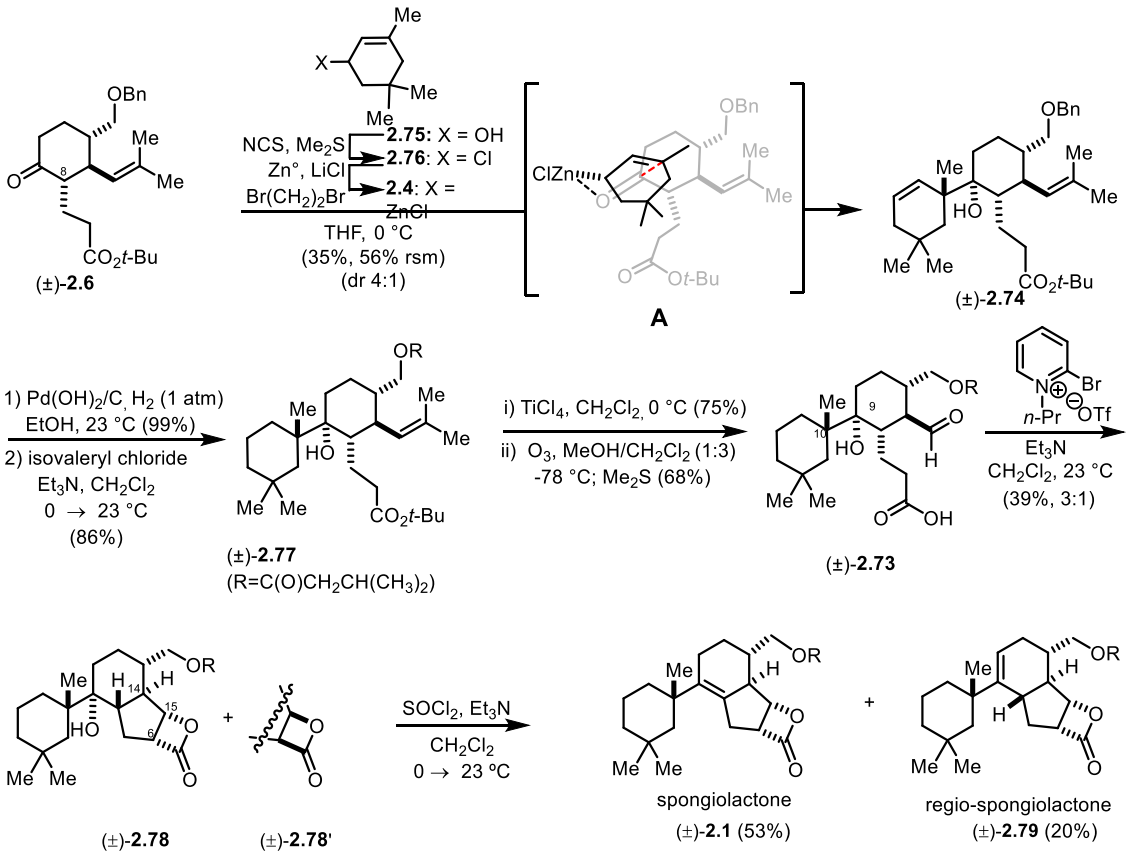


entry	equiv of **2.4**	temperature (°C)	[reaction]	time (h)	Result
1	1.2	-78	0.5 M	2	Rsm
2	1.2	-20	neat	12	Rsm
3	1.9	-20	neat	12	Rsm
4	3.6 over 24 h	-78 → 23	0.06 M	18	Rsm
5	1.2	-78 → 23	0.01 M	24	Rsm
6	1.2	-78 → 23, 30→ 65	0.2 M	6	double add'n

Following extensive optimization studies, we ultimately found that *in situ* generation of the allyl zinc reagent (±)-**2.4** in the presence of ketone (±)-**2.6** led to the greatest chemoselectivity and conversion. Under these conditions, using a large excess

of zincate, the desired tertiary alcohol (±)-**2.74** bearing two vicinal quaternary centers was obtained as an inseparable 4:1 mixture of diastereomers in 24-35% yield (Scheme 2.17).

Scheme 2.17



While yields are modest, ketone (±)-**2.6** (45-60%) could be easily recovered and recycled. Some degree of facial selectivity is anticipated for the major diastereomer of (±)-**2.6**, as shown for one enantiomer of both ketone and zincate, proceeding through cyclic transition state **A**, as previously proposed for related additions to

cyclohexanones.[36, 42] This transition state arrangement predicts the major diastereomer for new stereocenters C9 and C10 as shown in alcohol (±)-**2.74** which was verified following subsequent transformations leading to a crystalline intermediate.

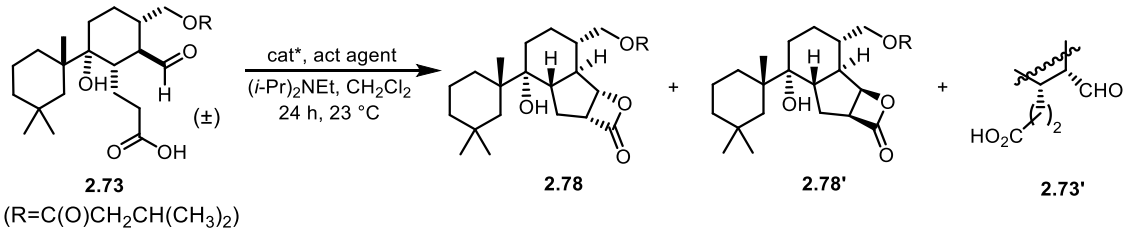
Hydrogenation with Pearlman's catalyst enabled chemoselective reduction of the cyclohexene with concomitant hydrogenolysis of the benzyl ether of (±)-**2.74** delivering a primary alcohol which was directly esterified with isovaleryl chloride to provide ester (±)-**2.77**. The latter intermediate was crystalline and enabled verification of relative stereochemistry by single crystal X-ray analysis. Conditions to selectively cleave the *t*-butyl ester over the isovaleric ester were identified employing $TiCl_4$[45], and subsequent ozonolysis afforded racemic aldehyde acid (±)-**2.73**.

With this aldehyde-acid in hand, we were eager to subject this structurally complex substrate to our NCAL methodology. *In situ* activation of the carboxylic acid with modified Mukaiyama's reagent[24] along with triethylamine as both the nucleophilic promoter (Lewis base) and Brønsted base pleasingly provided desired product (±)-**2.78** and its diastereomeric β-lactone (±)-**2.78'** in a 3:1 ratio, respectively. The final step toward spongiolactone involved dehydration of tertiary alcohol (±)-**2.78**. This was accomplished using thionyl chloride to afford spongiolactone (±)-**2.1** along with the regioisomeric alkene, regio-spongiolactone (±)-**2.79** in a ~2.7:1 ratio favoring the more substituted alkene found in the natural product (Scheme 2.19). Characterization data for synthetic spongiolactone correlated well with data previously reported for both spongiolactone[21] and nor-spongiolactone.[17]

2.2.4 NCAL-Based Kinetic Resolution: Accessing (+)-Spongiolactone and Derivatives

We first studied a racemic NCAL involving *in situ* activation of the carboxylic acid with the previously described modified Mukaiyama's reagent[24] using triethylamine as both the nucleophilic promoter (Lewis base) and Brønsted base. This enabled determination of the inherent diastereoselecitivty of the NCAL with aldehyde acid (±)-**2.73** and provided samples for chiral HPLC analysis. Under racemic NCAL conditions, two β-lactone diastereomers, **2.78** and **2.78'**, were produced in modest yield as a 3:1 mixture of diastereomers (Table 2.3, Entry 1). Commercially available TsCl was also a competent activating agent, however modified Mukaiyama's reagent provided the best yields (Entry 2 vs 3). Premised on our recent success employing isothiourea catalysts for the NCAL process with keto acid substrates including homobenzotetramisole (HBTM) developed by Birman,[46] we first studied both optical antipodes of HBTM. (*S*)- and (*R*)-HBTM in combination with the modified Mukaiyama's reagent delivered the enantiomeric β-lactones (+)- and (–)-**2.78** in 6-16% yields (maximum yield 45% for kinetic resolution employing (±)-**2.73** with 4:1 dr) in up to 78-84% ee (Entries 4,5) along with 12-16% of the diastereomeric β-lactones **2.78'**. Importantly, (*R*)-HBTM also reversed the inherent diastereoselectivity of the substrate (Entry 1 vs 5, 3:1 vs 1:2 dr). The enantiopurity of the undesired diastereomeric β-lactone **2.78'** was low (20 % ee) with both promoters, however (-)-benzotetramisole (BTM) improved the enantiopurity of the undesired diastereomer (+)-**2.78'**, but did not improve the enantiopurity of (+)-**2.78** (Entry 5).

Table 2.3 Optimization of the kinetic resolution *via* the NCAL process with (±)-**2.73**.[a]



entry	Lewis base	yield [%] (**2.78:2.78':2.73'**)[b]	dr (**2.78:2.78'**)[c]	*ee* [%] (**2.78**)[d]	*ee* [%] (**2.78'**)[d]
1	Et_3N	30:9:ND	3:1	-	-
2[e]	(±)-HBTM	10:10:ND	1:1	-	-
3	(±)-HBTM	16:2.5:ND	6:1	-	-
4	(*S*)-HBTM	16:16:ND	1:1	78[f]	20[f]
5	(*R*)-HBTM	6:12.5:ND	1:2	84	20
6	(-)-BTM	11:10:ND	1.2:1	81	60
7	TMSQD	19:14:27	1.3:1	93[f]	77[f]
8	TMSQN	10:4:34[g]	2.5:1	90	ND
9[h]	TMSQN	10:3:18[g]	5:1	92	ND
10	TMSQN[i]	10:<5:25[g]	2:1	90	ND

[a] All reactions were performed under the following conditions: 1.0 equiv nucleophilic promoter, *N*-^{n}Pr-2-bromopyridinium triflate (modified Mukaiyama's reagent, 3.0 equiv), (^{i}Pr)$_2$NEt (5.0 equiv), CH_2Cl_2, 24 h, 23 °C unless noted otherwise. [b] Yields refer to isolated purified yields. [c] Diastereomeric ratios determined by integration of ^{1}H NMR (500 MHz) or by isolation of each diastereomer (entries 1-3, 8, 9). [d] Enantiomeric excess was determined by chiral phase HPLC. [e] TsCl (1.5 equiv) was used as the activating agent. [f] Enantiomeric excess for the enantiomeric products, (–)-**2.78'** and (–)-**2.78'**. [g] Yield of **2.78'** estimated from ^{1}H NMR (500 MHz) integration. [h] 2.0 equiv of (^{i}Pr)$_2$NEt was used. [i] 0.27 equiv of catalyst employed. ND = Not Determined.

We next studied *Cinchona* alkaloid derivatives which had previously proved successful with aldehyde acid substrates.[22] Use of TMSQD (Entry 6) and TMSQN (Entry 7) showed marked improvement in enantioselectivity, with both promoters

providing ≥ 90% *ee* in 15-33% yields along with significant quantities of epimerized aldehyde (±)-**2.73'** (27-34%). Following conversion to the natural product (*vide infra*), we determined, by comparison of optical rotations, that TMSQN generated the enantiomer (+)-**2.78** required for synthesis of (+)-spongiolactone. Resubjecting the epimerized aldehyde to the NCAL conditions showed that this diastereomer did not undergo the NCAL process suggesting that the relative stereochemisty of β-lactones **2.78, 2.78'** differed at the β-lactone rather than C14 and this was subsequently confirmed by conversion of β-lactone **2.78** to spongiolactone and detailed NMR analysis of (+)-**2.78'** and (±)-**2.73'**.[i] Toward minimizing epimerization of the substrate aldehyde (±)-**2.73**, the equivalents of Hünig's base were descreased. This decreased epimerization and also improved the diastereoselectivity of the reaction providing a 5:1 dr (Entry 8). While our initial studies made use of a full equivalent of chiral promoter, it should be noted that decreasing the catalytic loading to 27 mol% did not decrease the yield nor enantioselectivity but did have an impact on diastereoselectivity (Entry 9).

2.3 Conclusion

In summary, we completed the first total synthesis of (+)-spongiolactone **2.1** in 11 steps from commercially available 1,3-cyclohexanedione. The synthesis highlights the use of an *in situ* generated, complex cyclohexenyl zincate for addition to a complex, sterically hindered ketone to generate two adjacent quaternary carbon stereocenters, including an all-carbon quaternary stereocenter as well as an advanced application of the NCAL process.

CHAPTER III

CYTOTOXICITY STUDIES OF (+)-SPONGIOLACTONE AND DERIVATIVES: DISCOVERY OF A MORE POTENT, UNNATURAL DERIVATIVE, REGIO, *BIS*-EPI SPONGIOLACTONE

3.1 Introduction

β-Lactones are effective enzyme acylating agents. Through covalent modification of nucleophilic active site residues, they have demonstrated pharmacological relevance in their inhibition of fatty acid synthase (FAS) and serine hydrolases.[47] Specific examples include, but are not limited to, orlistat (**3.1**), omuralide (**3.3**), and salinosporamide A (**3.5**).

Orlistat (tetrahydrolipstatin, **3.1**) is an FDA approved anti-obesity drug (Figure 3.1). Mechanistically, acylation of serine residues in the active sites of pancreatic and gastric lipases leads to irreversible inhibition of these enzymes, which are responsible for the conversion of triglycerides to absorbable monoglycerols and free fatty acids. As part of a separate screening for prostate cancer inhibitors, orlistat was also found to be a novel inhibiter of the thioesterase domain of fatty acid synthase, which plays a role in tumor growth progression.[48] X-ray crystallography confirmed that this inhibition results from acylation of the thioesterase molecules via the β-lactone moiety in the active site of FAS.[49]

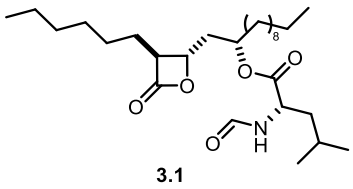


Figure 3.1 Orlistat: a known FAS inhibitor.

Omuralide (*clasto* lactacystin β-lactone, **3.3**), a β-lactone containing small molecule active against the 20S proteasome, arises from the natural product lactacystin *in situ* under slightly basic conditions (pH 8) through loss of *N*-acetylserine (Figure 3.2).[47] Mechanistically, omuralide leads to irreversible inhibition by acylation of the N-terminal threonine in the proteasome.

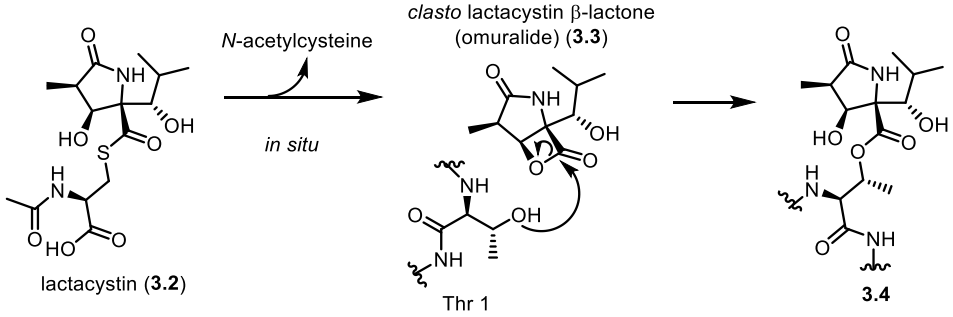


Figure 3.2 Mechanism of inhibition for lactacystin.

Salinosporamide A (**3.5**) is a marine natural product, discovered in 2003 by Fenical, Jensen and coworkers[50] that also inhibits the 20S proteasome by forming a covalent adduct with an N-terminal threonine (Figure 3.3). Structurally, the fused γ-lactam, β-lactone core is the same as that found in omuralide (**3.3**), however

salinosporamide A also contains a chloroethyl side chain, a methyl substituent at the β-lactone, and a cyclohexene in place of an isopropyl group. Its inhibitory activity against the 20S proteasome is at the nanomolar level and persists for longer than 24 hours. Mechanistically, this prolonged inhibition has been found to arise from formation of tetrahydrofuran (**3.7**) by an S_N2 displacement of the alkyl chloride by the alcohol that comes from the initial opening of the β-lactone (**3.5**).[51]

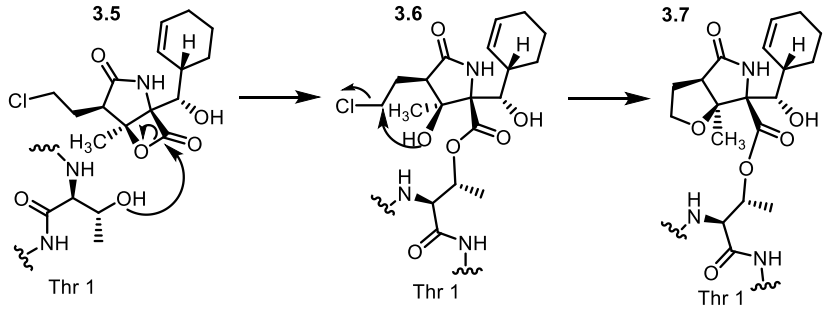


Figure 3.3 Inhibition of 20S proteasome by salinosporamide A.

The tetrahydrofuran introduces additional steric bulk around the ester adduct between the threonine and salinosporamide A (**3.5)**, effectively blocking its hydrolysis and thus reducing reversibility. Because of its remarkable activity, salinosporamide A entered Phase I clinical trials as a candidate to treat multiple myeloma.[52]

3.1.1 Targeted β-lactone Derivatives Based on Synthetic Route to Spongiolactone

Given the precedent for β-lactones acting as covalent inhibitors,[47] and the chemistry developed toward spongiolactone, regio-isomeric, *bis*-epimeric, and more

simplified tricyclic β-lactones were targeted to accompany spongiolactone for biological activity testing (Figure 3.4).

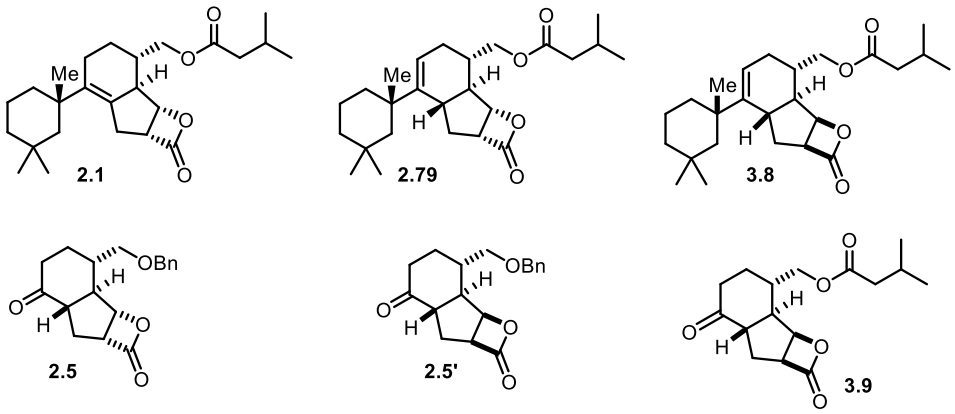


Figure 3.4 Targeted derivatives for biological activity testing.

All of these compounds could be accessed by chemistries developed toward the natural product itself and, in fact, compounds **2.1**, **2.79**, **2.5**, and **2.5'** had already been synthesized. Depending on the observed cytotoxicity of spongiolactone, we hypothesized that simplified tricyclic β-lactones **2.5** and **2.5'** could provide insight into the pharmacophore which was already thought to be, in part, the β-lactone moiety.

3.1.2 Measuring Cell Viability by the MTT Assay

When carrying out cytotoxicity studies, it is crucial to have an effective, reliable way to quantify the net effect of the compound of interest on cells. To do this, assays are often used where a colorimetric change takes place, brought about by the surviving cells. Tetrazolium salts, which are pale in color, have found great utility on these

grounds, given their conversion to vibrant formazens by dehydrogenase enzymes (Figure 3.5), which are only active in living cells.[53, 54] The tetrazolium salt 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT) has proved especially useful for this application. The amount of formazen produced directly correlates with the number of living cells and the colorimetric assay of the sample can be carried out directly without any type of purification or washings.[53] Further, this method is generalizable across cell lines.

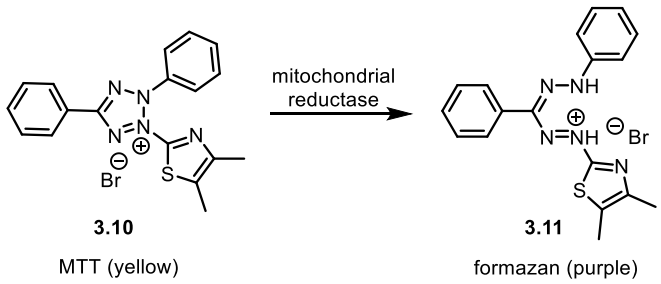


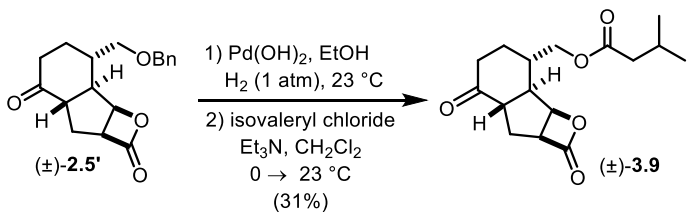
Figure 3.5 Conversion of MTT to formazen in living cells.

3.2 Results and Discussion

3.2.1 Synthesis of Derivatives

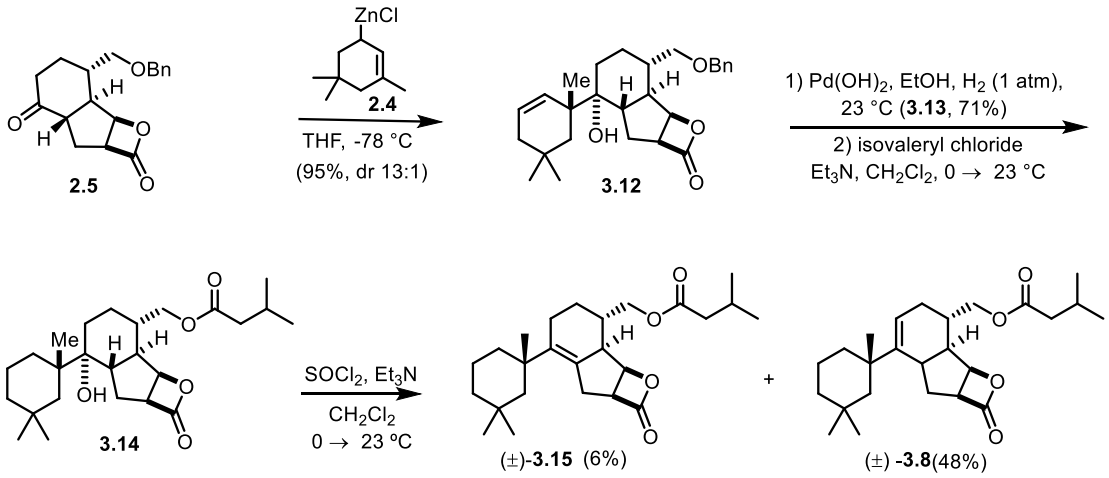
The first additional derivative we targeted was simplified tricyclic β-lactone **3.9** that would contain the same isovaleric ester side chain that is found in the natural product (Scheme 3.1). Starting from β-lactone **2.5'**, hydrogenolysis using Pearlman's catalyst cleanly provided the intermediate primary alcohol, which was immediately acylated with isovaleryl chloride to give the isovaleric ester derivative **3.9**.

Scheme 3.1



The second additional derivative synthesized was regioisomeric, *bis*-epimeric spongiolactone. Utilizing the allyl zinc addition adduct that had been previously synthesized in our investigations into the S_E' addition to tricyclic β-lactone **2.5**, Pearlman's catalyst was employed to reduce the cyclohexene with concomitant hydrogenolysis of the benzyl ether to provide an intermediate primary alcohol (**3.13**, Scheme 3.2) which was immediately acylated with isovaleryl chloride to yield isovaleric ester **3.14**. Dehydration with thionyl chloride led to regioisomeric olefin products: tetrasubstituted olefin **3.15** and tri-substituted, 6,15-*bis*-epi-spongiolactone **3.8**.

Scheme 3.2

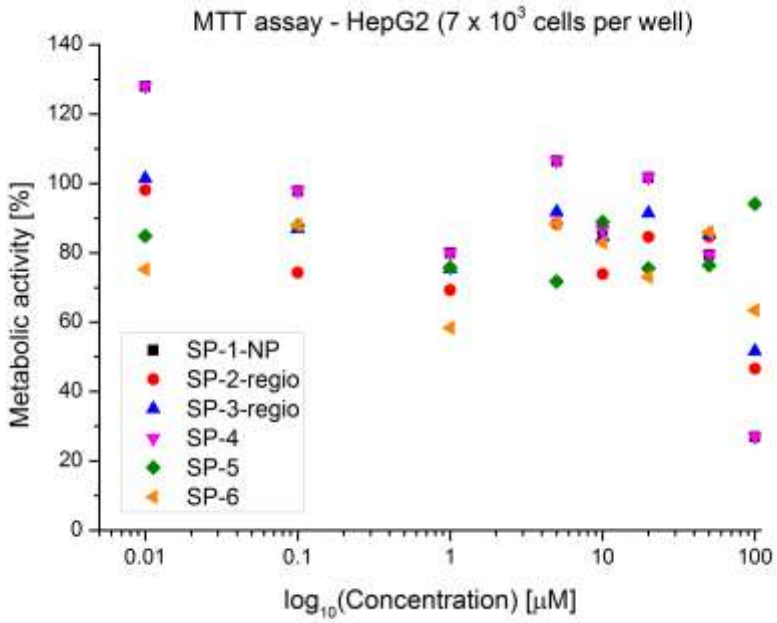


3.2.2 Initial Screening Against Various Cancer Cell Lines

Initial screening of (+)-spongiolactone (**2.1**) and its derivatives was carried out against HepG2-hepatocellular carcinoma (liver cancer), A549-lung adenocarcinoma (lung cancer), and MCF-7-breast adenocarcinoma (breast cancer) cell lines.[†] Disappointingly, no inhibitory activity was observed in of these cell lines, as determined by the MTT assay (Figure 3.6).

[†] In collaboration with Professor Stephan Sieber and research assistant Dr. Joanna Krysiak at Technische Universität München.

a.



b.

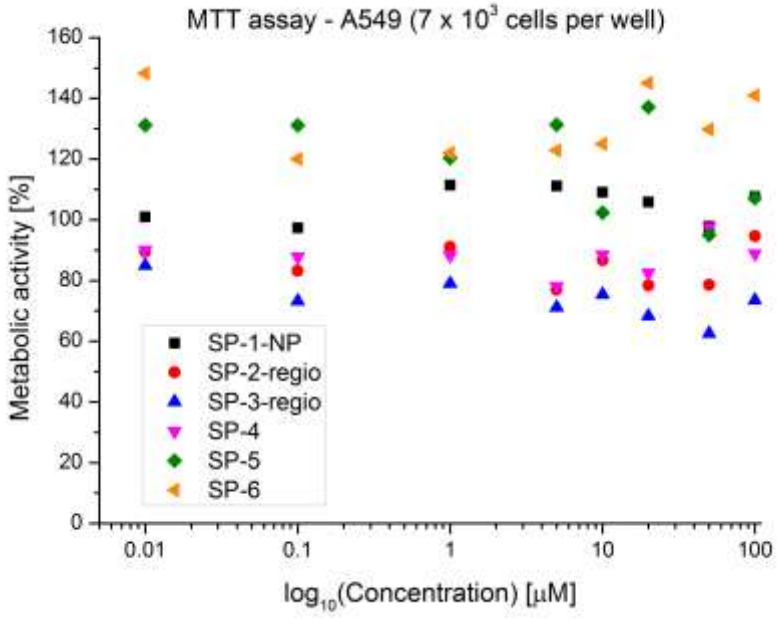


Figure 3.6 Summary of results for MTT assay conducted against **a.** liver cancer cells **b.** lung cancer cells and **c.** breast cancer cells.

c.

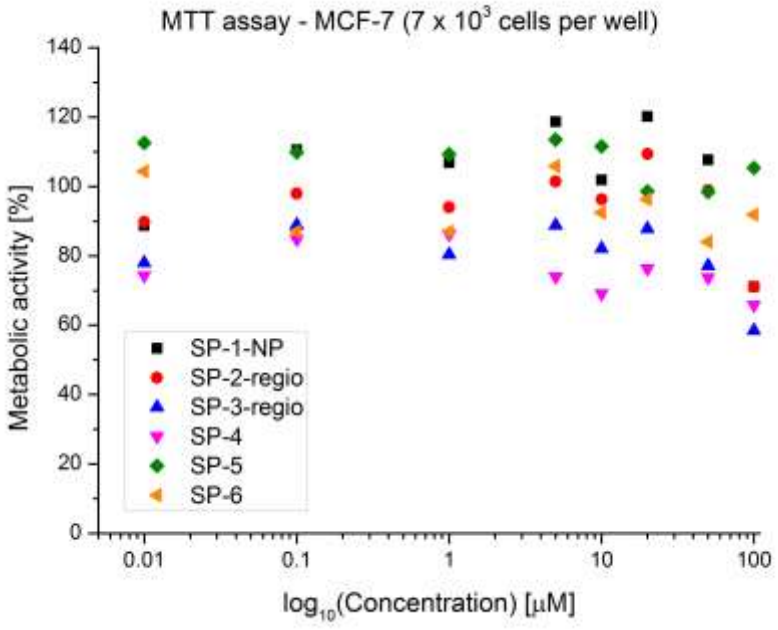


Figure 3.6 Continued.

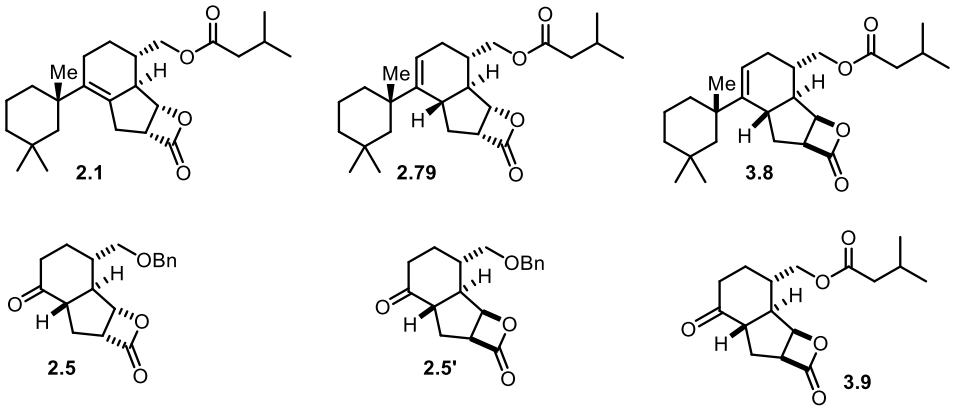
Here, it can be seen that even at increased concentrations of the derivatives, the % metabolic activity remains relatively constant.

3.2.3 Preliminary Cytotoxicity of Spongiolactone and Derivatives

Fortunately, screening of the K562 human myelogenous leukemia cell line, the same cell line in which the β-lactone congener of spongiolactone (**2.1**), nor-spongiolactone, showed much more promising results specifically for the natural product itself as well as the regio-isomeric, *bis*-epimeric-spongiolactone (**3.8**), Interestingly, the benzyl ether tricyclic β-lactones (**3.9** and **3.10**), as well as regio-isomeric spongiolactone (**2.79**), differing only by having a tri-substituted olefin instead of a tetra-substituted

olefin showed no inhibition, with IC_{50} values of >500 μM, even though the presumed pharmacaphoric β-lactone is present in all three derivatives (Table 3.1). These results imply that the overall topology of the molecule, which affects the molecule's ability to acylate a specific protein, is highly impacted by the olefin geometry, and that the pendant cyclohexane, and all-carbon quaternary center, are critical for achieving inhibition.

Table 3.1 IC_{50} values for (+)-spongiolactone **2.1** and derivatives against the K562 human chronic myelogenous leukemia cell line.



Entry	β-lactone[b]	IC_{50} ± SEM (μM) [b]
1	spongiolactone ((+)-**2.1**)	129 ± 10
2	regio spongiolactone ((+)-**2.79**)	>500
3	regio, *bis*-epi spongiolactone ((±)-**3.8**)	29 ± 4
4	benzyl ether (±)-**2.5**	>500
5	diastereomeric benzyl ether (±)-**2.5'**	>500
6	isovaleric ester (±)-**3.9**	297 ± 49

[a] Optically active material was accessed employing TMSQN as a nucleophilic promoter and simplified β-lactones were accessed by aldehyde acid (±)-**2.71**. [b] All IC_{50} values were determined by utilizing a MTT metabolic activity assay. Assays were performed in triplicate. SEM = standard error of the mean.

The observed cytotoxicities for **2.1** and **3.8** were shown to be reproducible, with **2.1** (spongiolactone) having an average IC_{50} value of 132 μM. We were intrigued to find that the unnatural derivative **3.8** had much higher inhibition, with an average IC_{50} value of 29 μM for a racemic sample. If we hypothesize that only one enantiomer is leading to the observed inhibition, then the IC_{50} value for the active enantiomer should be much lower.

3.3 Conclusion

In conclusion, spongiolactone **2.1** and five other novel derivatives were submitted for cytotoxicity studies. The natural product along with its regio-isomeric, *bis*-epimeric analogue showed cytotoxicity against the K562 cell line. Of note is that the IC_{50} value for the unnatural analogue **3.8** was more potent than the parent natural product.

CHAPTER IV

CELLULAR TARGET IDENTIFICATION OF (+)-SPONGIOLACTONE AND REGIO, *BIS*-EPI SPONGIOLACTONE

4.1 Introduction

Upon identification of bioactive natural products through screening various cell lines, it is desirable to identify the specific protein target of the small molecule. Once target identification is complete, the small molecule can be modified to improve activity, or, alternatively, to identify other simplified structures that can produce the same effect. There are five common strategies employed in cellular target identification, including high-throughput screening, phenotype-based approaches,[55, 56] identification by deduction, affinity chromatography, and activity-based protein profiling[55] (ABPP). Of these, affinity chromatography and ABPP have received considerable attention, with both relying on modification of the molecule of interest with a tag and subsequent binding to a protein.

4.1.1 Activity-based Protein Profiling (ABPP) and Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) for Cellular Protein Target Identification

Activity-based protein profiling (ABPP) is used to study enzyme subsets within a given proteome. It relies on covalent modification of an electrophilic, small molecule probe by nucleophile addition of an amino acid residue specifically located at or near the binding pocket of the molecule within a protein target[57]. Aside from an electrophilic

reactive group, the probe also contains a spacer and a reporter tag. The reporter tag provides a means for a bio-orthogonal coupling reaction,[58] such as a 'click' reaction, which involves a copper(I)-catalyzed alkyne-azide [3+2] cycloaddition through a step-wise modification of the Huisgen triazole synthesis.[59] In this case, the reporter is a terminal alkyne[60] (Figure 4.1). The labeled proteome is subjected to click chemistry conditions with an azide coupling partner, which contains either a fluorophore or biotin.

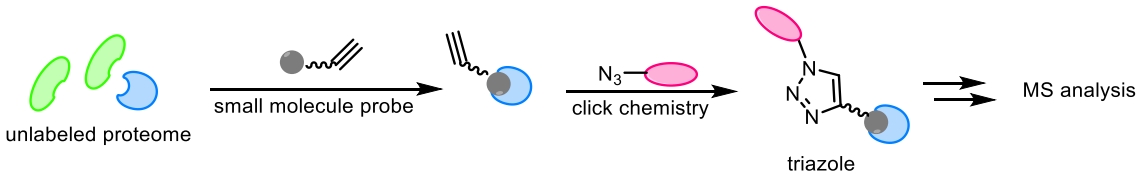


Figure 4.1 General schematic of ABPP.

Once the triazole is formed, the probe-labeled protein is separated from the proteome by either gel electrophoresis (if a fluorophore is present) or by affinity separation using streptavidin beads. The protein(s) can be identified by subsequent digestion from the bead followed by mass spectrometry analysis. While this approach has proved effective in target identification, it does not allow the level of inhibition to be quantified.

Stable isotope labeling by amino acids in cell culture (SILAC) is a mass-spectrometry technique that enables quantitative proteomic analysis through utilizing isotopically labeled arginine or lysine (Figure 4.2).[61] This technique has been coupled

with ABPP as a direct and sensitive way to quantify inhibitory effects of small molecule probes.

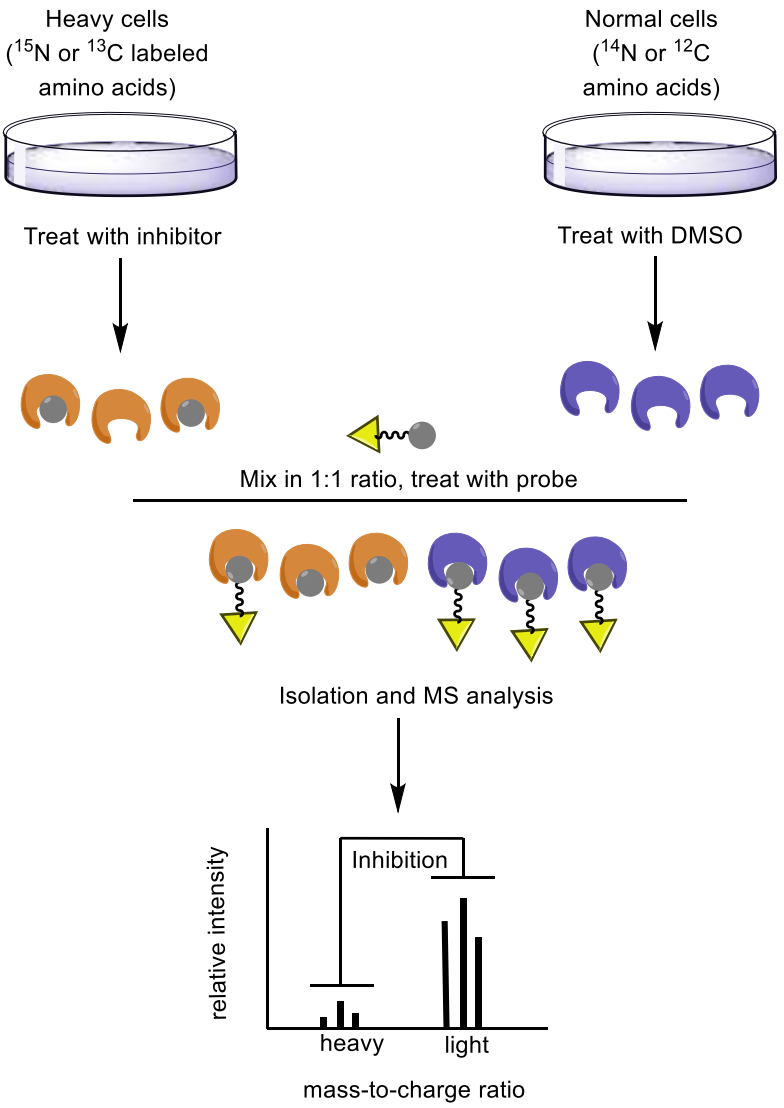


Figure 4.2 Stable isotope labeling by amino acids in cell culture (SILAC) coupled with ABPP.

In this approach, two batches of the cells of interest are grown, where one sample will be fed either ^{13}C or ^{15}N (heavy) labeled amino acids and the other sample will be fed

^{12}C or ^{14}N (light) amino acids. Over several divisions, the amino acids become incorporated into the cells. It should be noted that whether heavy or light amino acids are used, there are no behavioral differences between the cells. Either the light or heavy cells are then treated with an inhibitor, while the other is treated with DMSO to serve as the control. A biotinylated probe is then added to both sets of cells and the previously described method for ABPP is carried out. The samples from the different cell media are combined and by mass spectrometry the relative protein abundance quantified by examining the relative intensities of the two sets of peaks resulting from the isotopically labeled samples. Protein identification is also possible in this approach by peptide fragment analysis via MS-MS.[62]

4.1.2 Synthetic Design of an Alkyne-Containing Probe

In considering the synthetic design of an alkyne-containing probe, it is critical for the spacer and alkyne to have a negligible effect on the interaction between the reactive group of the probe and the enzyme active site. Thus, the spacer should not have significant steric bulk, impart rigidity, or alter the hydrophobicity/hydrophilicity of the compound.

The reactive group of the probe should be of sufficient electrophilicity such that it can undergo nucleophilic attack by amino acid residues such as serine and cysteine. Many ubiquitin derivatives have been successful in studying deubiquitinating enzymes[57] (Figure 4.3 a). Other more common electrophiles, such as fluorophosphonates **4.6** have demonstrated high specificity for serine hydrolases (Figure 4.3 b), and epoxides, such as

natural product E-64 (**4.9**) have been utilized extensively with cysteine proteases[63] (Figure 4.3 c).

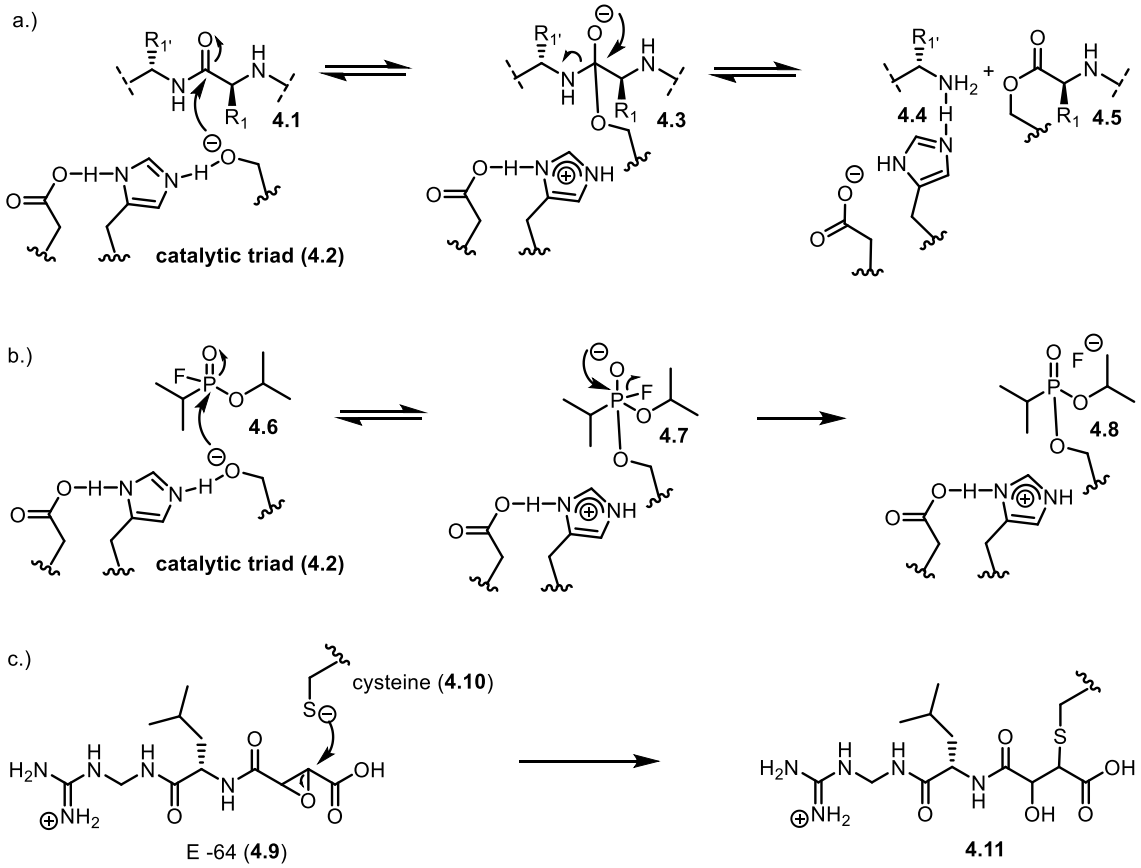
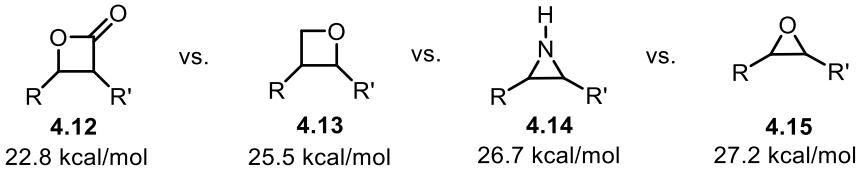


Figure 4.3 a.) Ubiquitin probes (**4.1**) for deubiquitinating enzymes, b.) fluorophosphonate probes (**4.6**) for serine hydrolases, and c.) epoxide probes (**4.9**) for cysteine proteases.

Other reactive groups include Michael acceptors (i.e. α, β-unsaturated carbonyls), alkyl halides (such as activated alkyl fluorides), and acylating groups (such as β-lactams and β-lactones).

4.1.3 β-lactones and ABPP

Over the past several years, β-lactones have emerged as effective small molecules for labeling enzymes. With a ring strain of approximately 22.8 kcal/mol, β-lactones (**4.12**) display moderate electrophilicity relative to oxetanes (**4.13**), aziridines (**4.14**), or epoxides (**4.15**) (Figure 4.4).



4.12 22.8 kcal/mol | **4.13** 25.5 kcal/mol | **4.14** 26.7 kcal/mol | **4.15** 27.2 kcal/mol

Figure 4.4 Comparison of ring strains relative to β-lactones.

In considering an enzyme active site, this modulated reactivity leads to greater selectivity for nucleophilic addition of an amino acid residue. The resulting covalent adduct is suitable for ABPP studies.

Not only can libraries of these molecules be prepared in a facile manner, but also the selectivity for different enzyme classes can be modulated by varying the R-group substituents. To this end, Sieber and coworkers have demonstrated the remarkable ability of relatively simple β-lactones to specifically label a wide variety of enzymes within several different families (Figure 4.5).[64]

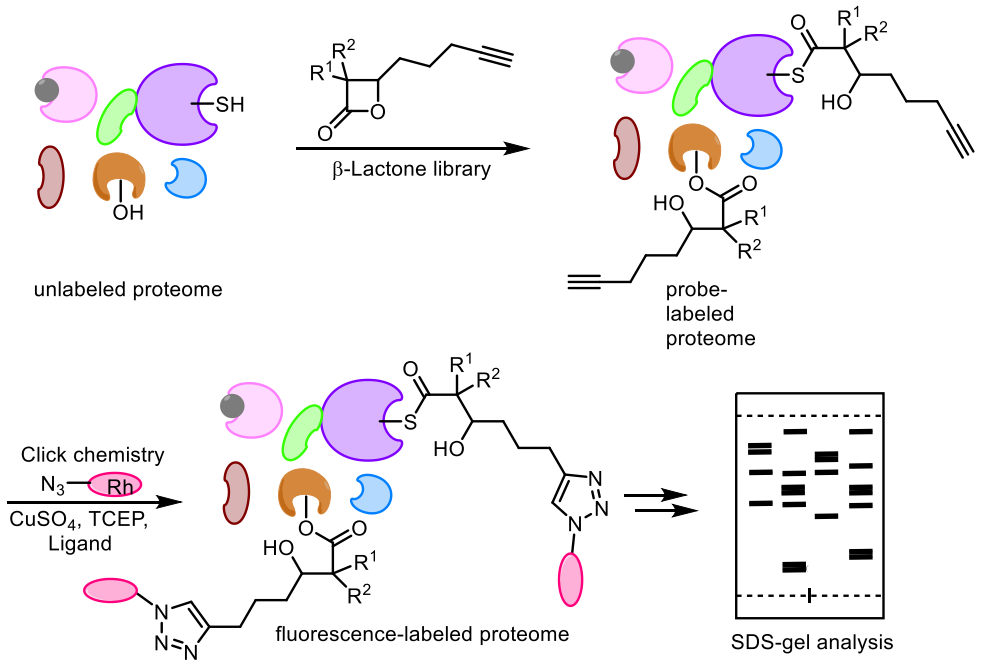


Figure 4.5 Application of β-lactone library to ABPP.

Through synthesis of a β-lactone library, and applying the ABPP technique, over twenty different enzymes were labeled from four major families.

4.2 Results and Discussion

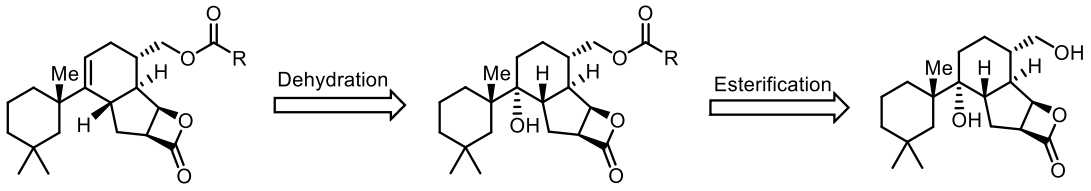
4.2.1 Total Synthesis of an Alkynyl Regio, *bis*-epi Spongiolactone Probe

Due to their complex scaffolds, which often translate into selectivity among different enzyme classes, natural products containing an electrophilic moiety are particularly appealing. However, installation of an alkyne onto a natural product for subsequent bio-orthogonal reactions can be a challenging task.[65] The natural product must either contain a functional group that is amenable to modification, or alternatively, the parent structure must be modified to enable the desired transformation. Such

structural revisions are often accessible through an established total synthesis. Given the demonstrated ability of simplified β-lactones to selectively label enzymes,[66-68] we were encouraged to pursue the synthesis of an alkyne-containing probe derivative from spongiolactone through only minor modifications to our established synthetic route to the natural product.

In considering the design of an alkyne probe derived from regio, *bis*-epi spongiolactone, we identified the pendant ester to be an ideal point of modification. Here, alkyne probe **4.16** would come from dehydration of tertiary alcohol **4.17** that could arise from esterification of preceding primary alcohol **4.18** which is easily accessible based on our established synthetic work (Scheme 4.1).

Scheme 4.1



4.16
regio-*bis*-epi spongiolactone:
$R=CH_2CH(CH_3)_2$
alkynal probe: $R=(CH_2)_5CH$

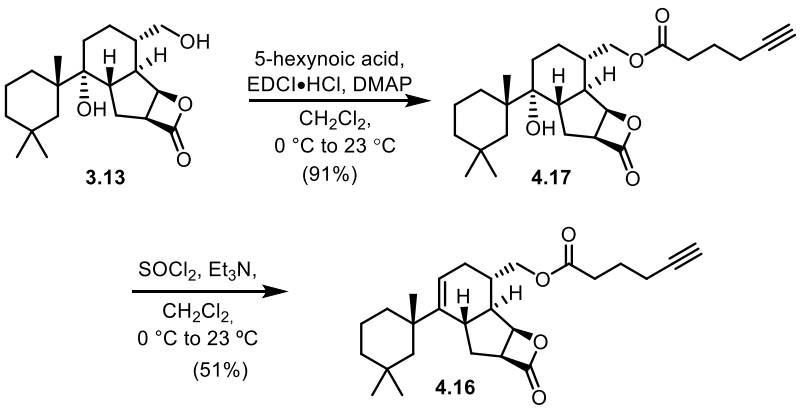
4.17
$R=(CH_2)_5CH$

3.13

To this end, primary alcohol **3.13** was treated under modified Steglich esterification conditions with 5-hexynoic acid to afford alkynyl ester **4.17** (Scheme 4.2).

This alkynyl acid was selected based on its moderate chain length and commercial availability. Subsequent dehydration provided **4.16** as an initial probe for ABPP studies.

Scheme 4.2



4.2.2. Verifying Cytotoxicity of Probes Through Cell Based Assays: MTT

Once a probe is synthesized, it is critical to confirm that this new derivative maintains the bioactivity observed for the parent compound. Ideally, the modification is not near the pharmacaphore and the activity remains nearly the same, if not the same. To this end, alkynyl probe **4.16** was used to treat K562 cells and the outcome evaluated by the MTT assay.

From this assay, run as a side-by-side comparison with **3.8**, we were pleased to find no significant change in cytotoxicity, with the probe having an IC_{50} value of 32.3 μM compared to 39.8 μM observed for parent compound **3.8**.

4.2.3 Preliminary labeling studies toward ABPP

Alkyne probe **4.16** was utilized in the SILAC experiment of K562 cells (Figure 4.6). Here, we are looking for the appearance of new bands that have been treated with the probe versus the control cells which have only been treated with DMSO. From these experiments, 4 new bands appeared which were excised and analyzed by mass spectrometry to try to identify proteins specifically labeled by the probe (25, 40, 32, 98 kDa).

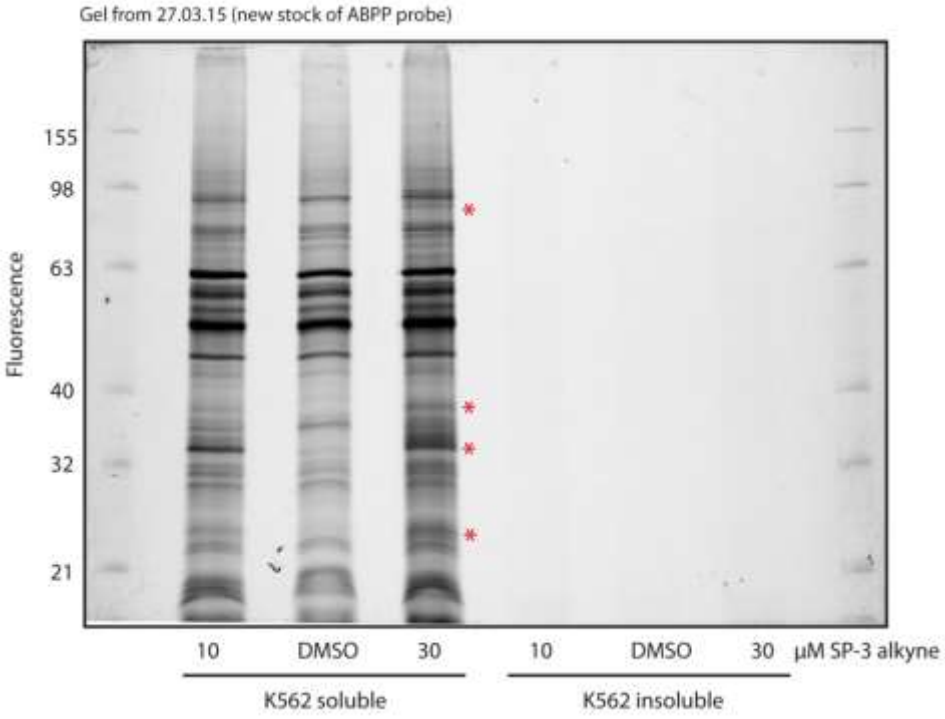


Figure 4.6 Gel from ABPP of K562 cells using alkynyl probe **4.16**.

From each band, a list of protein candidates was compiled including, but not limited to very-long-chain 3-oxoacyl-CoA reductase, heterogeneous nuclear ribonucleoprotein A0, ATP-dependent 6-phosphofructokinase, platelet type, casein

kinase II subunit beta, and heme oxygenase 2. Heme oxygenase 2 was one of the most intense bands observed. Further analysis is necessary to determine if this particular protein is a logical target for a β-lactone, given their typically observed inhibition by acylation.

4.3 Conclusion

Alkynyl probe **4.16** was successfully synthesized to be used in ABPP for target identification. Based on an MTT assay run in parallel with the parent compound of interest, no loss in cytotoxicity was observed against the K562 cell line. Further, preliminary ABPP with SILAC experiments showed the appearance of 4 unique bands which have been analyzed by mass spectrometry to compile a list of potential protein targets. Further experimentation is necessary to complete these studies.

CHAPTER V

RECYCLABLE ISOTHIOUREA CATALYSTS

5.1 Introduction

Organocatalysis can be defined as an acceleration brought about by the substoichiometric addition of an organic compound to a given reaction.[69] Described as the 'golden age' of organocatalysis, from 2004-2005, over 500 publications specifically addressing stereoselective organocatalysis appeared in the literature[70] and since this time, the remarkable potential of this field continues to be demonstrated. While more advancements in transformations amenable to organocatalysis are being developed, there are still areas for improvement.

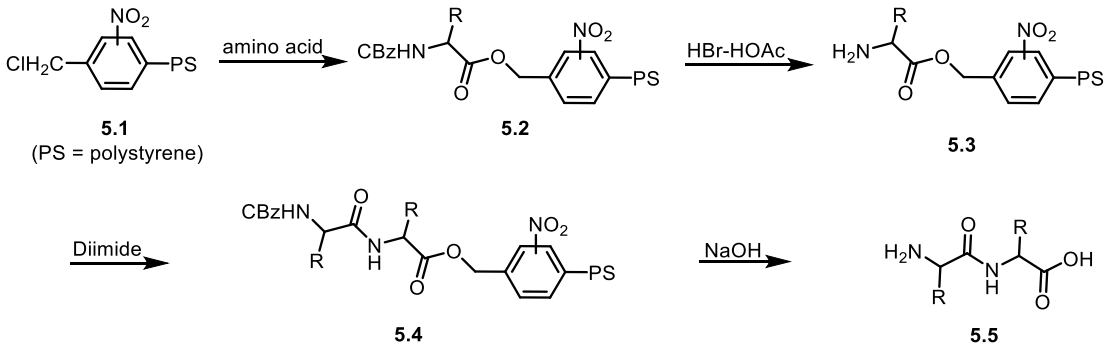
For example, the bar for increasingly lower catalytic loading continues to be raised as the catalysts themselves are often costly. Further, separation of the organocatalyst from the reaction product can often be tedious. To overcome cost and purification limitations, many chemists have turned to supported organocatalyst systems. Such developments seek catalyst recyclability, where the organocatalyst is able to be both recovered from the reaction and, subsequently, reused.

5.1.1 Immobilizing Organocatalysts: Solid versus Soluble Supports

Since the seminal report of solid-phase peptide synthesis (SPPS) by Merrifield in 1963,[71] work that earned him the Nobel Prize in Chemistry in 1984, the breadth of supported systems in chemical synthesis continues to grow. In Merrifield's approach, an

amino acid is covalently attached to a resin, the peptide chain is grown, and cleavage of the original linkage provides the free polypeptide **5.5** (Scheme 5.1).

Scheme 5.1



A few years later, Letsinger and coworkers applied a similar strategy to oligodeoxyribonucleotides with a styrene based, insoluble polymer as the support.[72] Inspired by this approach, much of the early work in catalyst supports utilized insoluble supports. In fact, the first example of supported chiral ligands by Kagan and co-workers[73] utilized cross-linked polystyrene beads (Merrifield resin) in the asymmetric, catalytic reduction of ketones. The use of solid supports was appealing as it allowed the catalyst to be separated from the reaction medium by simple filtration, but was limited in that the behavior of the immobilized, heterogeneous catalyst typically performed inferiorly to the free, homogeneous catalyst; an observation largely attributed to blocked active sites by the support itself.[74]

In an effort to achieve both excellent catalyst behavior and facile separation, Bayer and Mutter developed the first example of a chiral ligand attached to a soluble polymer[75] which they used in the asymmetric hydroformylation of styrene. Initially focusing on linear polystyrene as the soluble support, their work was later extended to include polyacrylonitrile, and poly-(ethyleniminodiacetic acid), polyethylenimine, and poly(vinylpyrrolidinone) to chelate metal catalysts.[76] Still, much of the early work utilizing soluble polymer supports faced problems in catalyst behavior and recovery.[77] This was observed by Bayer and coworkers in their hydrogenation work,[76] where the reaction times were 30-50% longer and isolation of the supported catalyst had to be done by either emulsion or ultrafiltration. In the time that has passed since these early reports of solid and soluble supported chemistries, many improvements have been made in both areas providing useful tools for recovering and reusing organocatalysts.

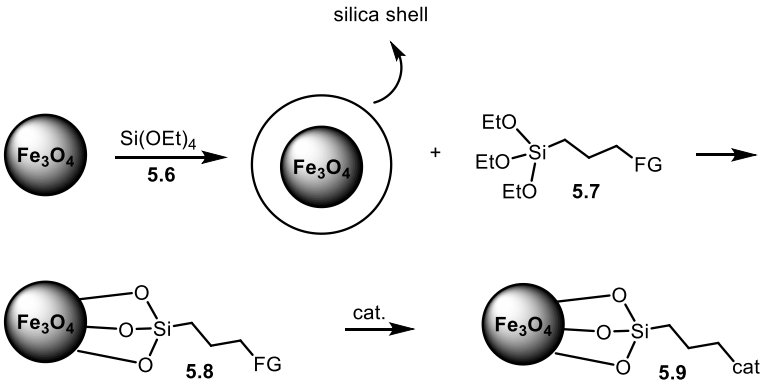
5.1.2 Magnetic Nanoparticles (MNPs) as Solid Supports for Organocatalysts

As an attempt to achieve the best of both worlds in supported organocatalysts, namely the facile separation of solid supports and increased reactivity of organocatalysts on soluble supports, magnetic nanoparticles have emerged as pseudo-heterogeneous supports.[74] Here, the supported organocatalyst is easily separated by applying an external magnet, avoiding the need for filtration or centrifugation as is typical in other solid supported systems. Additionally, when well-dispersed in the reaction media, their high surface area allows them to behave similarly to the soluble free catalyst.

The magnetic nanoparticles (MNPs) used for organocatalysts may be metal based (i. e. Ni, Fe, or Co), metal oxides ($MnFe_2O_4$, $CoFe_2O_4$, $CuFe_2O_4$, and Fe_3O_4), or alloys ($CoPt_3$, CoFe, or FePt).[78] While each type of nanoparticles has been successfully employed as organocatalyst supports, magnetite (Fe_3O_4) has emerged as an exceptionally useful support. Not only is the synthesis of magnetite cheap and facile, but it also is nontoxic and inert under a variety of conditions.[79]

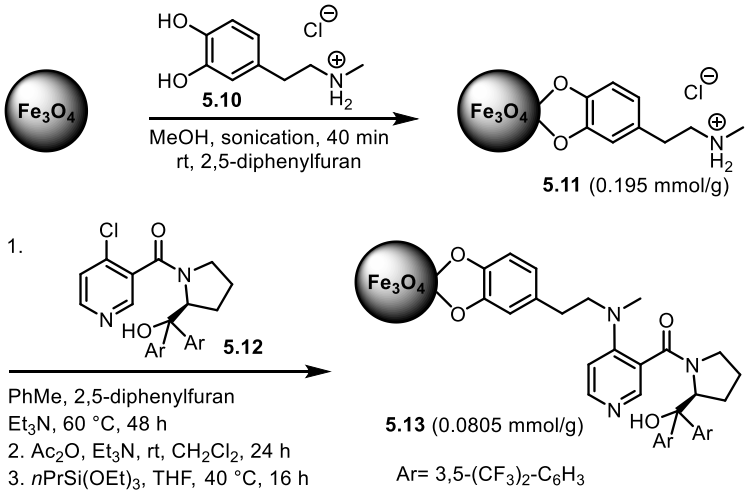
The organocatalyst itself can either be attached to the MNP by a linker or directly to the surface. Organosilicon linkers are especially popular, where a silica shell is formed on the surface of the magnetite by using tetraethoxysilane **5.6** and subsequent treatment with a functionalized triethoxysilane **5.7** provides a terminal functional group onto which the organocatalyst can attach (Scheme 5.2).[80]

Scheme 5.2



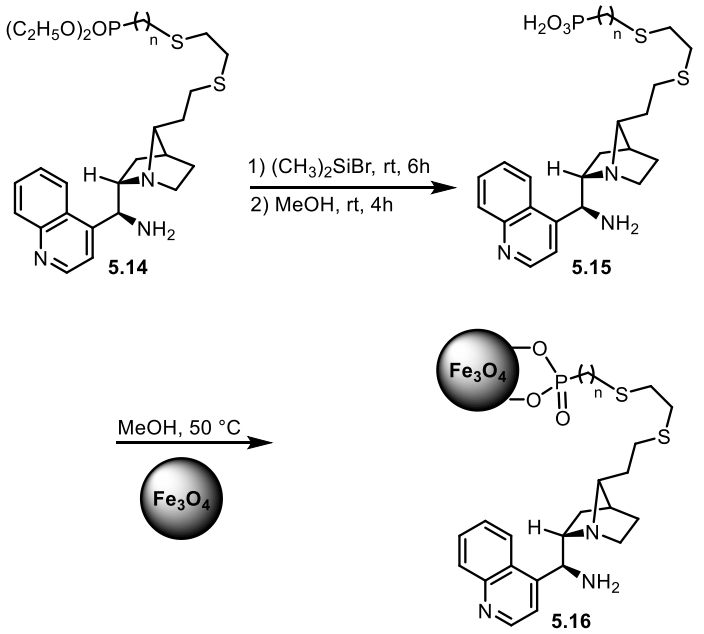
If instead direct attachment is desired, the organocatalyst must contain, either inherently or through derivatization, an appropriate functional group that will adhere to the magnetite surface. Catechol has been found to adhere strongly to the surface, making dopamine a commonly used linker for organocatalyst attachment.[81] Connon and coworkers utilized dopamine to develop the first magnetic nanoparticle supported chiral DMAP analogue (Scheme 5.3).[82] This catalyst was able to be recycled up to 30 times in the acylation of racemic *sec*-alcohols.

Scheme 5.3



Likewise, phosphonic acids are a stable means of attachment and, like dopamine, can be adsorbed onto the surface of magnetite (Scheme 5.4).[83]

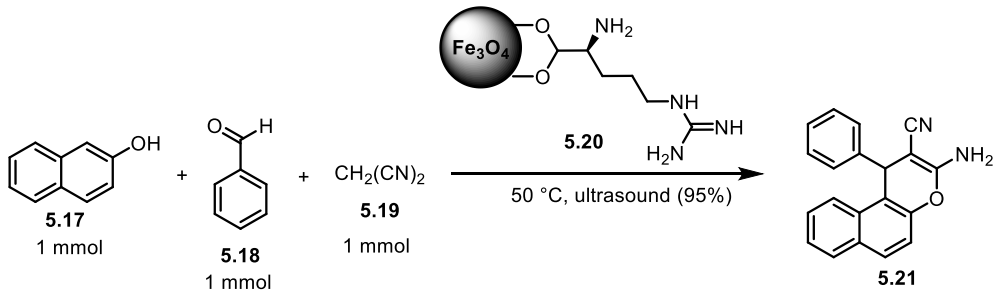
Scheme 5.4



In this example, the phosphonic acid **5.15** was revealed from the corresponding phosphonate ester **5.14** and allowed the cinchonidine-derived organocatalyst to be attached to the MNP. This catalyst system was applied in the asymmetric aldol reaction and could be recycled up to seven times.

Terminal carboxylic acids have also been used,[84] though this linker is less robust and more sensitive to the pH of the reaction medium. For example, amino acids such as arginine have been adsorbed onto magnetite directly and applied to the synthesis of chromenes, though the recyclability was limited to four cycles (Scheme 5.5).

Scheme 5.5



MNPs have proven successful in the area of organocatalyst supports, though they are not always inert in reactions and, in some cases, even coating the surface with siloxanes does not prevent a background reaction from taking place.[85]

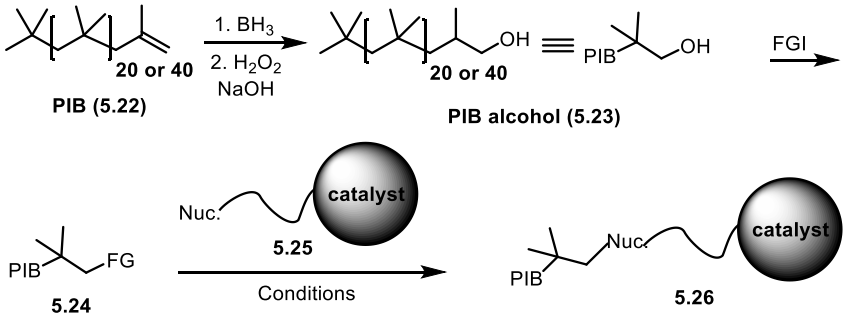
5.1.3 Polyisobutylene (PIB) as a Soluble Polymer Support for Organocatalysts

In the realm of soluble polymer supports, poly(alkene)oxide (PEG) has been used extensively.[86] However, while successful in serving as a support for a variety of catalysts, there are several limitations in using this polymer including solubility issues (the catalyst-supported systems are only completely soluble above 75 ˚C in nonpolar solvents and minimally soluble at any temperature less than 50 ˚C).[87] Considering that the vast majority of catalysts are used to carry out stereoselective reactions, which are often run at temperatures below 0 ˚C, PEG is not a viable option for a soluble polymer support.

PIB provides an alternative to PEG without the temperature-dependent solubility limitations.[88] Further, PIB provides excellent phase discrimination between polar and

non-polar solvents, making its recovery facile, and standard functional group interconversions of the terminal olefin allow this support to be tailored to accommodate a variety of nucleophiles as linkers to the catalyst (Scheme 5.6).

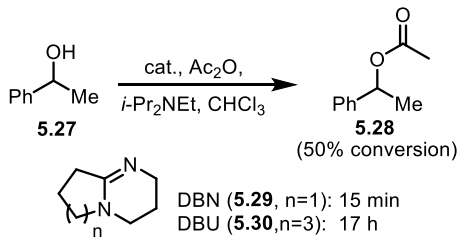
Scheme 5.6



5.1.4 Utility of Isothiourea Catalysts in Organic Transformations

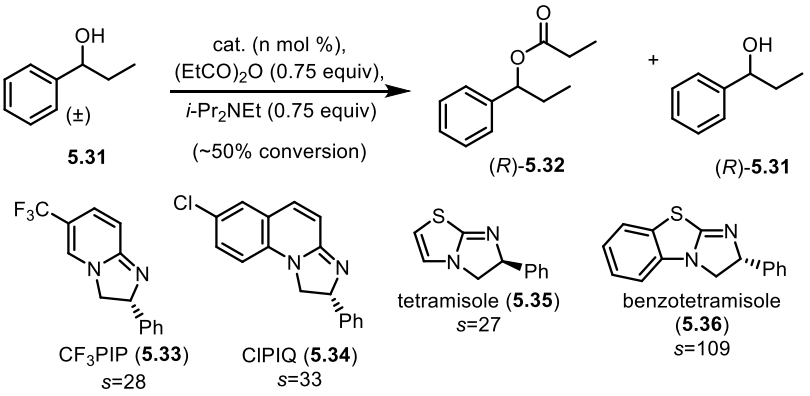
Amidine bases have long been used in organic synthesis. Though classically it is taught that common amidines, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) are non-nucleophilic bases, studies in organocatalysis have only recently uncovered the value of these systems as nucleophiles attributed to charge delocalization between the two nitrogen atoms.[89] Through probing the ability of various amidine systems to catalyze the acylation of simple alcohols, Birman and coworkers concluded that the size of the ring system impacted reactivity: DBU (**5.29**, a 7-6 bicyclic system) was ineffective in affording the desired transformation while DBN (**5.30**, a 6-5 system) worked well (Scheme 5.7).[90]

Scheme 5.7



With this information, they embarked on exploring a variety of ring sizes for amidine systems and isothioureas (Scheme 5.8). At the same time, Okamoto *et al* were also investigating similar modifications.[91]

Scheme 5.8

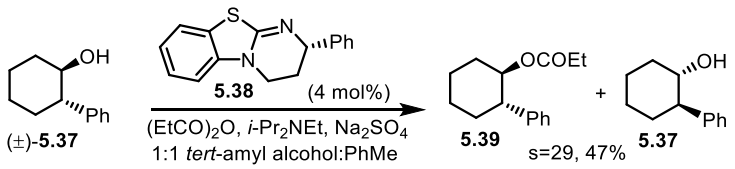


Ultimately, they arrived at benzotetramisole (BTM, **5.36**), which displayed superior selectivity in the kinetic resolution of secondary alcohols. Here, selectivity is a

measure of the ratio of reaction rates for each enantiomer of a given substrate.[92] It is calculated as $s = \ln[(1-ee_{sm})(1-c)]/\ln[(1+ee_{sm})(1-c)]$ where the conversion is defined as $c = ee_{sm}/(ee_{sm} + ee_{pr})$.

Since the development of BTM (**5.36**), homobenzotetramisole (HBTM, **5.38**) has also been introduced as an acylation catalyst for the kinetic resolution of aryl cycloalkanols (Scheme 5.9).[46]

Scheme 5.9



HBTM displayed greater activity than standard amidine systems which is attributed to added stabilization from the σ* of the sulfur atom with a nonbonding pair of electrons from the oxygen of the acylammonium.[93] Since its first introduction, various derivatives have been prepared,[94,95] that, along with HBTM itself, have been popularized in kinetic resolutions of secondary alcohols,[96] β-lactams,[97] annulation processes,[98] and formal [4 + 2] cycloadditions.[99] Further, these catalysts have seen extensive applications in acylammonium/ammonium enolate chemistry with new methodology that is fluorishing in the Romo group.[29]

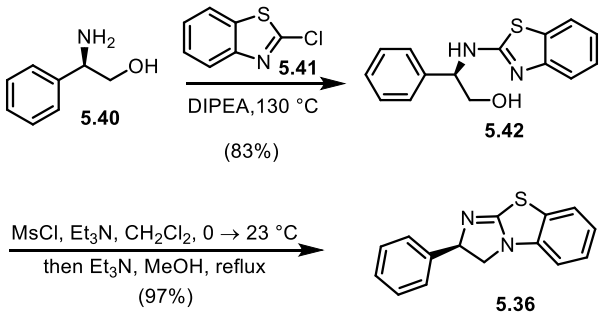
While HBTM and derivatives are excellent catalysts to achieve high enantioselectivity, a few disadvantages include solubility issues which limit reaction conditions, as well as their preparation, where attaining enantiopure material is costly. Though in theory the cost can be mitigated through catalyst recovery, it has proven very difficult to achieve experimentally. To circumvent these problems, use of an organocatalyst support is a viable option.

5.2 Results and Discussion

5.2.1 Synthesis of Magnetite-supported BTM

Based on the known preparation of benzotetramisole (Scheme 5.10), we recognized that either the 2-chlorobenzothiazole **5.41** or amino alcohol **5.40** would have to be modified.

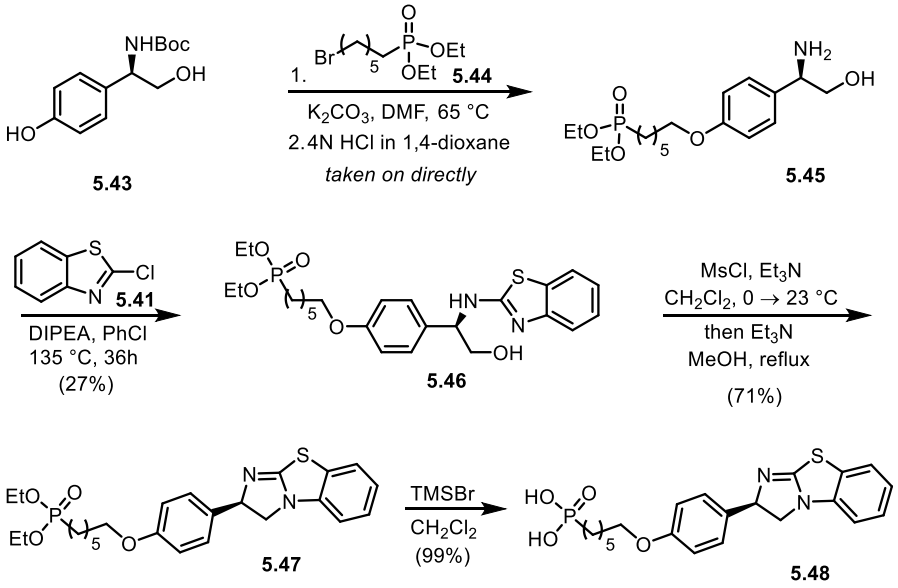
Scheme 5.10



Due to concerns that modification of the benzothiazole portion of the catalyst may alter its behavior, efforts were focused on modifying the amino alcohol. Fortunately, phenolic amino alcohol **5.43** is a known compound and was used as the starting material for our synthesis (Scheme 5.11).[100]

Desiring to utilize phosphonic acid groups to adsorb onto the magnetite, we used known bromo-phosphonate ester **5.44**[101] as an electrophile for the phenol. This reaction proceeded cleanly and was taken on directly to the boc-deprotection to provide free amino alcohol **5.45**.

Scheme 5.11

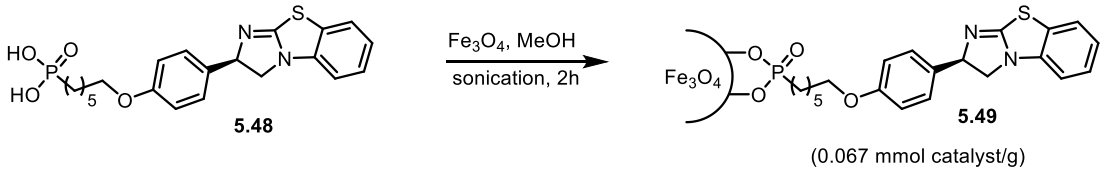


Amino alcohol **5.45** was then subjected to an S_NAr reaction with 2-chlorobenzothiazole **5.41** to give **5.46**. Subsequent mesylation of the primary alcohol

followed by cyclization gave phosphonate ester BTM derivative **5.47** for the first time. Since it is necessary to have the phosphonic acid instead of the phosphonate ester to enable adsorption onto the magnetite surface, the ethyl ester had to be cleaved. Here, trimethylsilyl bromide was found to be a suitable reagent to carry out this transformation and give the terminal phosphonic acid **5.48**.

With the catalyst in hand, we turned our efforts to adsorption onto the magnetite surface. The standard approach is to either sonicate or reflux a solution of the catalyst in the presence of magnetite.[82, 83] To this end, simple sonication (Scheme 5.12) in methanol provided the adsorbed catalyst **5.49** which was confirmed by IR spectroscopy, showing both the key stretches of the BTM catalyst (1612, 1570, and 1466 cm^{-1}) as well as the phosphonic acid adsorbed onto the magnetite (2600 cm^{-1}).

Scheme 5.12



Further, transmission electron microscopy (TEM) was employed to analyze **5.49** (Figure 5.1). Here, one can observe the magnetite core (larger than 8 nm in size) and a thin shell (~1 nm thick) on the surface which represents the adsorbed catalyst.

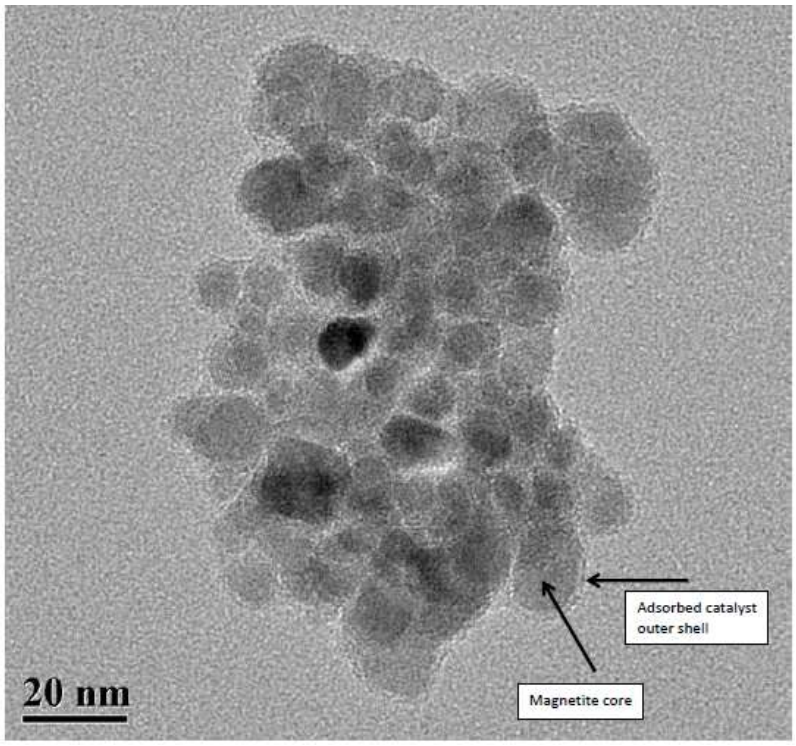


Figure 5.1 TEM image of phosphonic acid-(+)-BTM adsorbed on magnetite.

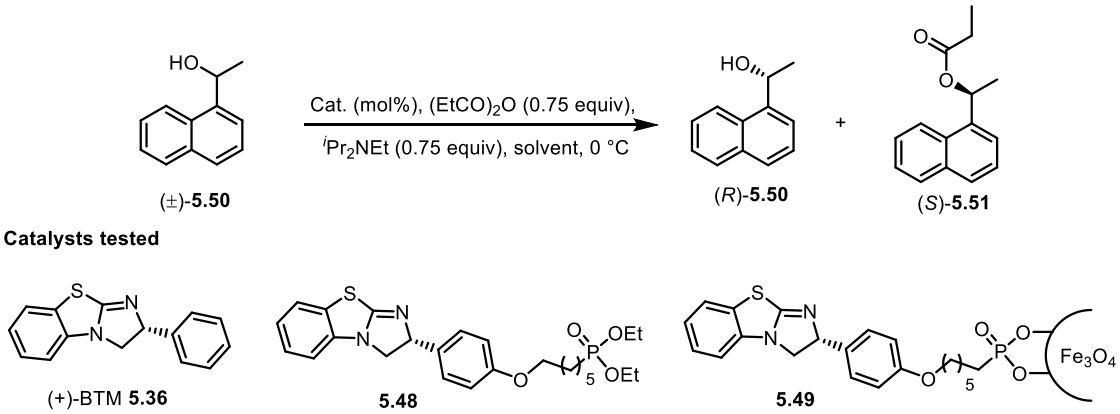
The loading of the catalyst onto the MNP was determined to be 0.067 mmol catalyst/1 g of sample by quantitative ^{31}P NMR spectroscopy. Having confirmed and quantified the catalyst loading onto the MNP, it was time to study its behavior relative to the parent, soluble (+)-BTM catalyst. We focused on the kinetic resolution of secondary alcohols; a reaction in which (+)-BTM is known to perform well. We chose 1-naphthylethanol as a starting substrate, as (+)-BTM is known to resolve this alcohol with high selectivity in under 12 hours (Table 5.1, Entry 1), and screened (+)-BTM, phosphonate ester **5.48**, and the absorbed catalyst **5.49**.[96]

In our hands, we found the best result for (+)-BTM to be achieved by Entry 3, stopping the reaction at 6 hours achieving a selectivity of 148. With the baseline

established for the homogeneous catalyst, prepared phosphonate ester derivative **5.48** was next put to the test. Under reaction conditions identical to Entry 3, **5.48** did not perform as well as the parent catalyst, however it should be noted that these conditions were not optimized for the new catalyst and that easily separable ester **5.51** was still able to be achieved in excellent enantioselectivity (Entry 4).

Next, we began our studies on the adsorbed catalyst **5.49**. Running the reaction in chloroform formed ester **5.51** in excellent enantiomeric excess (Entry 6, 94%), however the conversion was still only 15% over 18 hours. Extending the reaction time led to negligible improvements in conversion up to 40 hours. In an effort to overcome the sluggishness of the reaction, the catalytic loading was increased from 8 mol% to 20 mol%, however there was no improvement in conversion (Entry 7). Raising the catalyst loading further to 30 mol% saw a slightly faster conversion (Entry 8), but further increases to 40 mol% (Entry 9) and 100 mol% (Entry 10) gave no further improvements, with 100 mol% catalyst failing to give any conversion. At this point, the amount of loaded MNP required to achieve such high catalytic loadings resulted in a thick slurry, making the reaction medium difficult to stir and, ultimately, magnetic decantation highly impractical.

Table 5.1 Summary of kinetic resolution studies of 1-naphthylethanol with **5.49**.



entry	cat (mol%)	time (h)	solvent	$ee_{\mathbf{5.50}}$ (%)	$ee_{\mathbf{5.51}}$ (%)	c	s
1*	**5.36** (4)	9	PhMe	-	-	49	128
2	**5.36** (4)	8	$CHCl_3$	80	99	55	49
3	**5.36** (4)	6	PhMe	44	97	31	148
4	**5.36** (4)	8	PhMe	59	97	38	96
5	**5.48** (4)	3	PhMe	50	94	35	44
6	**5.49** (8)	18	$CHCl_3$	17	94	15	63
7	**5.49** (20)	18	PhMe	13	93	12	48
8	**5.49** (30)	7.5	PhMe	13	93	12	48
9	**5.49** (40)	20	PhMe	20	92	18	26
10	**5.49** (100)	81	PhMe	0	N/A	0	N/A

Table 5.1 Continued.

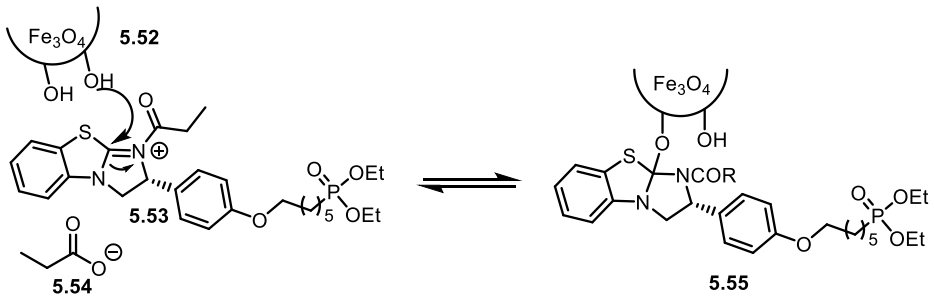
entry	cat (mol%)	time (h)	Solvent	$ee_{\mathbf{5.50}}$ (%)	$ee_{\mathbf{5.51}}$ (%)	c	s
11	**5.48** (4) + mnp	3	PhMe	30	95	24	52
12	**5.48** (4) + mnp	9	PhMe	43	93	32	33
13	**5.48** (4) + mnp	18	PhMe	58	92	39	37

*Reported results from literature;[96] $ee_{\mathbf{5.50}}$ and $ee_{\mathbf{5.51}}$ not reported.

While **5.49** did provide enantioenriched ester **5.51**, we sought to understand what was limiting the reaction conversion. To test whether the nanoparticles themselves were playing a role in hindering the reaction, free phosphonate ester was used in the presence of bare magnetite. The amount of magnetite added was equal to the amount of **5.51** that would have been added on the same reaction scale. After 3 hours, the observed results (Entry 11) were nearly identical to **5.48** (Entry 5), though even at this point the conversion was lower. Allowing the reaction to proceed for longer showed the rate of conversion and selectivity dropping off significantly (Entries 12 and 13). The conversion did not increase further after 18 h.

These crude results seem to point to an interaction taking place between the catalyst and the MNP, especially considering the results with the bare MNP (Entries 11-13) where the reaction initially proceeds the same as the free catalyst **5.48** but then the reactivity diminishes. One possibility is that the catalyst is deactivated by free hydroxyls on the MNP surface (Scheme 5.13). Similar deactivation has been reported for (+)-BTM in the presence of moisture during the kinetic resolution of secondary alcohols.[96]

Scheme 5.13

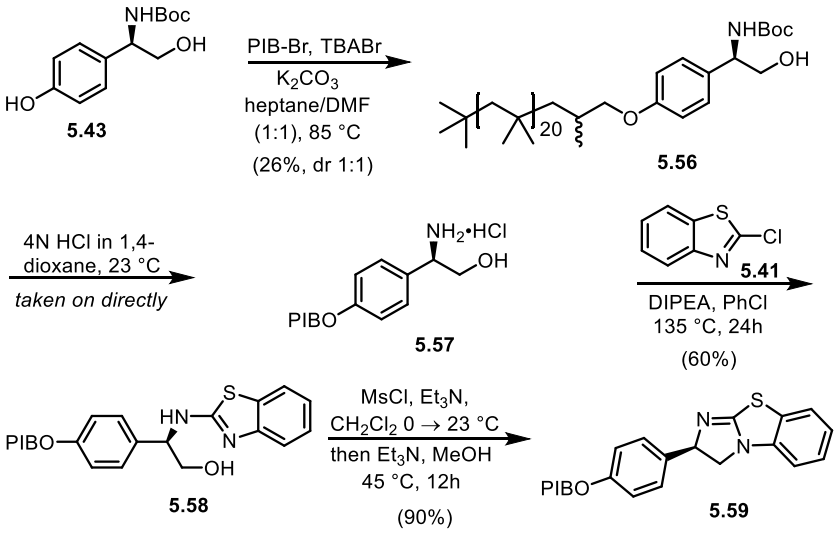


Unable to push the reaction to a useful conversion, and with the seemingly detrimental effect of magnetite on the reaction, the focus was switched from a solid-supported catalyst to a soluble, polymer-supported catalyst. Specifically, we turned our efforts to synthesizing a polyisobutylene (PIB) derivative of (+)-BTM.

5.2.2 Synthesis of PIB-supported BTM

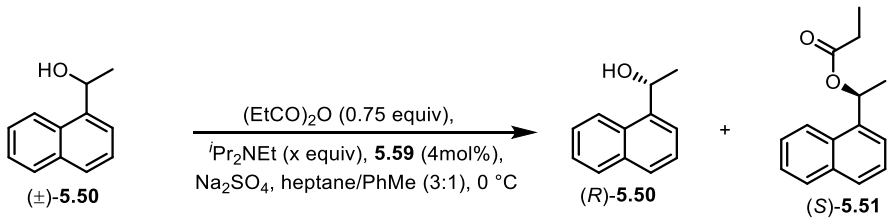
Beginning with phenol **5.43**, alkylation with previously prepared PIB-bromide[88] in the presence of tetrabutylammonium bromide (TBABr) as a phase transfer catalyst provided sufficient quantities of PIB-phenol **5.56** (Scheme 5.14). Boc-deprotection mediated by 4N hydrochloric acid in 1,4-dioxane proceeded quantitatively and the resulting amino alcohol hydrochloride salt **5.57** was taken on directly to the S_NAr reaction with 2-chlorobenzothiazole **5.41**. Mesylation of primary alcohol **5.58** followed by cyclization provided PIB-(+)-BTM derivative **5.59**.

Scheme 5.14



With the PIB-(+)-BTM catalyst **5.59** in hand, we first sought to measure its behavior relative to parent catalyst (+)-BTM **5.36** in the kinetic resolution of secondary alcohols (Table 5.2).

Table 5.2 Initial studies of **5.59** for kinetic resolution of 1-(1-naphthyl)ethanol **5.50**.



entry	time (h)	$ee_{\mathbf{5.50}}$ (%)	$ee_{\mathbf{5.51}}$ (%)	c	s
1	7.5	56	94	37	74
2	4	99	81	55	49
3	2.5	92	90	50	79
4*	2.5	97	88	52	76

*Na_2SO_4 excluded from reaction.

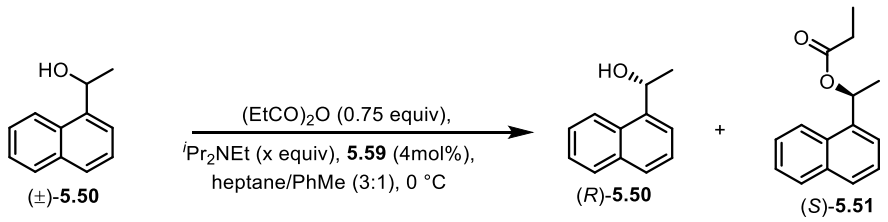
Though overall the selectivity for **5.59** was inferior to **5.36**, the reaction was much faster, reaching 50% conversion in only 2.5 hours (Entry 3). Further, both secondary alcohol **5.50** and ester **5.51** were achieved in excellent enantioselectivity. These two adducts are easily separable.

Having confirmed that **5.59** is a competent catalyst, studies turned to recoverability and recyclability. Initial attempts to use a biphasic solvent system of heptane and acetonitrile effectively separated the catalyst from starting alcohol **5.50**, however, given that **5.51** is highly nonpolar, a mixture of this product and the catalyst was attained in the heptane layer. Switching to DMF as the polar organic solvent showed the same results. During this experimentation, it was observed that if the reaction was directly concentrated and acetonitrile was added, **5.59** formed a separate

film that could be washed further with acetonitrile to give the catalyst. Unfortunately, even with these results, the highest recovery was 76%.

Toward recycling the catalyst, initial results were disappointing and gave very low conversion even after extended reaction time (Table 5.3, Entry 1). Here, recovered **5.59** was used directly. Reasoning that it may be necessary to account for the fact that the isolated catalyst was isolated as a salt from the reaction, the amount of Hünig's base was increased to 1.5 equiv (Entry 2). This dramatically improved the conversion, however the selectivity was still significantly less than the originally used **5.59** (s= 79), pointing to a significant background reaction taking place in the presence of excess base. Free-basing **5.59** prior to subjecting it to the reaction showed a reasonable rate of conversion though the selectivity was still low (Entry 3).

Table 5.3 Recyclability studies of **5.59** for kinetic resolution of secondary 1-(1-naphthyl)ethanol (**5.50**).



entry	time (h)	treatment of **5.59**	DIPEA (equiv)	$ee_{\mathbf{5.50}}$ (%)	$ee_{\mathbf{5.51}}$ (%)	C	s
1	16	-	0.75	4	92	4	51
2	4	-	1.5	64	92	41	47
3	2	free-base (DIPEA)	0.75	40	92	30	43
4	2	MeOH quench	0.75	48	92	34	46

Quenching the reaction with methanol, as is typically done in other kinetic resolution reactions,[96] did not lead to any improvement in recovery (62%) nor did it lead to improved catalyst behavior.

5.3 Conclusion

Benzotetramisole (BTM, **5.36**) was successfully adsorbed onto the surface of magnetite nanoparticles through synthesis of phosphonic acid derivative **5.48**. This marks the first isothiourea catalyst to be adsorbed to the magnetite surface. While the formed adduct was able to catalyze the kinetic resolution of secondary alcohol **5.50**, the highest selectivity achieved was 59 with 24% conversion. Due to separate experiments that pointed to catalyst deactivation taking place in the presence of the nanoparticles, the approach to recycling **5.36** was switched from a solid-supported magnetite catalyst to a soluble, PIB-supported catalyst.

PIB derivative **5.59** was successfully synthesized and showed good selectivity and conversion in the kinetic resolution of **5.50**. The most recent studies have examined recoverability and recyclability of this system. Further optimization is required in both of these areas. It should also be noted for both the magnetite derivative **5.49** as well as PIB-derivative **5.59** other reactions may be more suitable for these catalyst systems beyond the kinetic resolution of secondary alcohols.

CHAPTER VI

CONCLUSIONS

6.1 Total Synthesis of (+)-Spongiolactone and Subsequent Bioactivity Studies

The first total synthesis of (+)-spongiolactone was completed in 11 steps starting from commercially available 1,3 cyclohexanedione. The synthetic route featured a late-stage nucleophile-catalyzed aldol lacontization (NCAL) reaction on a highly advanced aldehyde acid substrate. Further, a novel allyl zinc reagent was developed to forge a challenging all-carbon quaternary center adjacent to a tertiary alcohol. This key transformation required the development of a one-pot protocol for the desired S_E' reaction on a tri-substituted cyclohexanone, pushing the limits for this type of addition with respect to substrate complexity.

Along with the natural product itself, five additional derivatives were prepared by modified chemistries developed in the total synthesis. Once the derivatives were prepared, various cancer cell lines were screened, with inhibitory activity observed for the K562 cell line. Most notable from this work was the discovery of a novel regioisomeric, *bis*-epimeric derivative more potent than spongiolactone itself. This derivative was used to form a biological probe containing a terminal alkyne that is being used in activity based protein profiling (ABPP) experiments.

6.2 Synthesis and Initial Studies of Supported-BTM Catalysts

Toward rendering isothiourea catalysts, namely benzotetramisole (BTM) recyclable, both a solid-supported, magnetite adduct as well as a soluble-supported,

polyisobutylene adduct were synthesized. Both displayed reasonable selectivity relative to the parent BTM catalyst, with the PIB-BTM catalyst performing superior to the magnetite system. Given this, along with experiments which suggest catalyst deactivation with magnetite, studies were directed toward the polymeric system.

Here, reaction conversion was faster than BTM with overall good selectivity. Improvements are still necessary regarding both recoverability and recyclability. Lastly, other transformations beyond the kinetic resolution of secondary alcohols should be explored to test the generality of the PIB catalyst and to see if better recyclability can be achieved.

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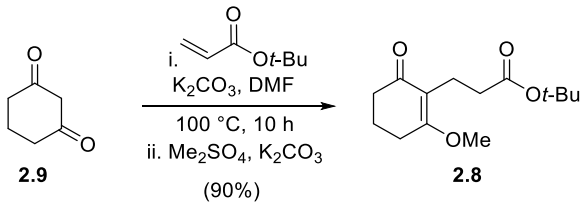
APPENDIX A

EXPERIMENTAL PROCEDURES

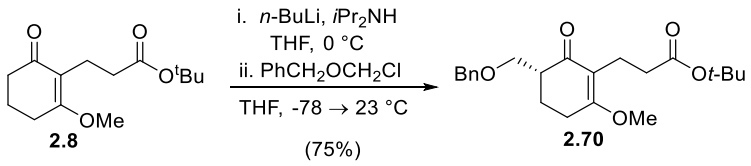
General Procedures

All reactions were completed under a nitrogen atmosphere in oven-dried glassware unless indicated otherwise. *N*, *N*- Dimethylformamide was dried over activated molecular sieves (4 Å). Dichloromethane was dried by passage through alumina or activated molecular sieves (MBraun solvent purification system). Tetrahydrofuran was freshly distilled over sodium and benzophenone. Methanol was freshly distilled from magnesium turnings. Triethylamine, Hünig's base, diisopropyl amine, and trimethylsilyl chloride were distilled from calcium hydride. Cerium chloride heptahydrate was flame-dried under vacuum. Commercially available reagents were used as received. Zinc powder was purchased from Strem Inc. (99.9%). ^{1}H NMR were measured at 500 MHz and chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (7.26 ppm,), coupling constants (J) are reported in Hertz (Hz), and multiplicity follows normal convention: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dddd, doublet of doublet of doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; ddq, doublet of doublet of quartets; ABq, AB quartet; m, multiplet; bs, broad singlet (prefix app indicates 'apparent'). ^{13}C NMR were measured at 125 MHz with deuterochloroform ($CDCl_3$) as the internal standard (77.16 ppm) for all ^{13}C spectra. Flash column chromatography was performed using 60Å silica gel (Silicycle, 230-400 mesh). Mass spectra (ESI) were obtained at the Center for Chemical Characterization and Analysis (Texas A&M University). Optical rotations were recorded on a polarimeter at 589 nm employing a 25 mm cell. High performance liquid chromatography (HPLC) was performed using 25 cm chiral columns as indicated. Thin layer chromatography was performed using glass-backed 60Å silica gel F-254 (Silicycle, 250 μm thickness). Developed plates were visualized by immersion in ceric ammonium molybdate stain followed by heating. Infrared spectra were obtained on a FTIR spectrometer as a thin film on NaCl plates. Transmission electron microscopy (TEM) was

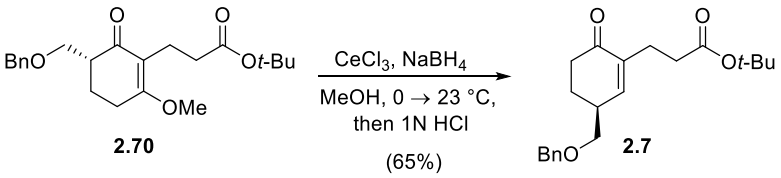
obtained by the Microscopy and Imaging Center at Texas A&M University utilizing a JEOL 2010 and micrographs were recorded at medium range magnification (71-145 kX). X-ray diffraction was obtained by the X-ray Diffraction Laboratory at Texas A&M University.



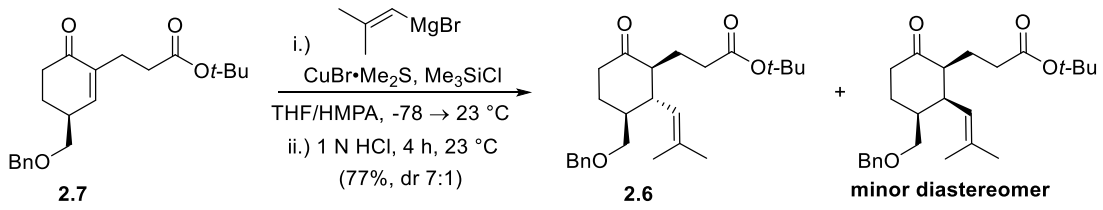
β-Keto enol ether 2.8. To a solution of 1,3-cyclohexanedione (12.0 g, 107 mmol, 1.0 equiv) in *N*, *N*- dimethylformamide (200 mL) was added K_2CO_3 (17.6 g, 127 mmol, 1.2 equiv). The heterogeneous solution was stirred for 10 min, resulting in a bright orange solution. *Tert*-butyl acrylate (18.4 mL, 127 mmol, 1.2 equiv) was added dropwise and the reaction was heated to 100 °C with stirring for 10 h. The reaction was then cooled to ambient temperature (23 °C) and a second portion of K_2CO_3 (17.6 g, 127 mmol, 1.2 equiv) was added followed by Me_2SO_4 (12.0 mL, 127 mmol, 1.2 equiv). Upon completion of the reaction, as indicated by TLC, quenching with pH 7 buffer solution (200 mL) gave a homogeneous solution which was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed sequentially with water (2 x 200 mL) and brine (200 mL), dried with Na_2SO_4 and concentrated *in vacuo*. The resulting dark brown, viscous oil was purified by filtration through a silica plug (SiO_2 30 → 60% EtOAc/hexanes) to give 24.5 g (90%) of β-keto enol ether **2.8**. TLC (EtOAc:hexanes, 1:1 *v*/*v*): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.78 (s, 3H), 2.53 (dt, *J* = 14.5, 6.0, Hz, 4H), 2.30 (t, *J* = 6.0 Hz, 2H), 2.21 (t, *J* = 8.5 Hz, 2H), 1.96 (quintet, *J* = 6.5 Hz, 2H), 1.40 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 197.3, 172.6, 172.3, 117.5, 79.3, 54.8, 36.0, 33.9, 27.8 (3), 24.5, 20.5, 17.3; IR (thin film): 3271, 2978, 1729, 1649, 1616, 1371, 1163, 1089 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{14}H_{22}LiO_4$ $[M + Li]^+$: 261.1678, found: 261.1683.



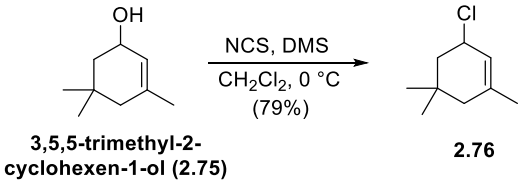
Benzyl ether 2.70. In a flame-dried round-bottomed flask, a solution of diisopropyl amine (4.3 mL, 30.5 mmol, 1.55 equiv) was prepared in THF (20 mL). The solution was cooled to 0 °C followed by dropwise addition of *n*BuLi (18.3 mL, 29.4 mmol, 1.49 equiv). Stirring continued for 30 min before cooling to -78 °C. A solution of **2.8** (5.0 g, 19.7 mmol, 1.0 equiv) in THF (10 mL) was added dropwise *via* syringe pump over 1 h. After stirring for an additional hour at -78 °C, benzyl chloromethyl ether (5.45 mL, 39.3 mmol, 2.0 equiv) was added neat, dropwise *via* plastic syringe. The reaction was warmed to ambient temperature (23 °C) and stirred for 12 h. Quenching was done at 0 °C with saturated NH_4Cl (20 mL). The organic phase was separated and the aqueous layer was further extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (60 mL), dried with Na_2SO_4, and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2 0 → 50% EtOAc/hexanes) gave 5.13 g (70%) of benzyl ether **2.70** as a yellow oil: TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.4 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37-7.30 (m, 5H), 4.52, 4.50 (ABq, J_{AB} =12 Hz, 2H), 3.89 (dd, J = 10.0, 4.5 Hz, 1H), 3.79 (s, 3H), 3.57 (app t, J = 9.0 Hz, 1H), 2.66 (dt, J = 18.0, 5.0 Hz, 1H), 2.55-2.46 (m, 4H), 2.29-2.20 (m, 3H), 1.89-1.81 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 197.1, 172.8, 172.0, 138.3, 128.2 (2), 127.54, 127.47, 127.4, 117.5, 79.5, 73.1, 69.7, 54.9, 44.8, 34.1, 28.0 (3), 23.9, 23.7, 17.8; IR (thin film): 3061, 3031, 2931, 1723, 1613, 1154 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{22}H_{31}O_5$ $[M + H]^+$: 375.2171, found: 375.2176.



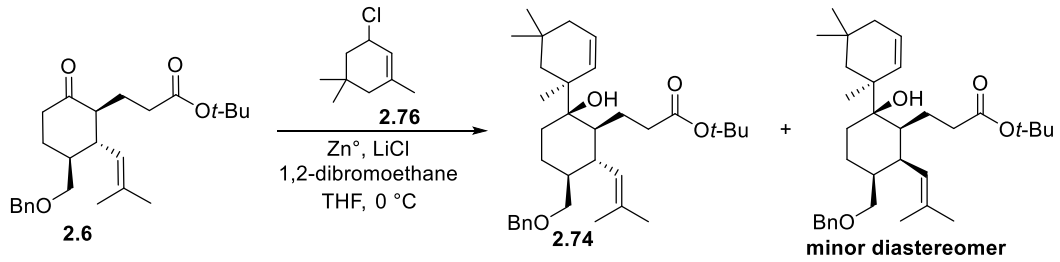
Cyclohexenone 2.7. A solution of **2.70** (5.13 g, 13.7 mmol, 1.0 equiv) in MeOH (140 mL) was prepared in an oven-dried round-bottomed flask. Cerium chloride (10.2 g, 41.4 mmol, 3.0 equiv) was added and dissolved over 10 min. The homogeneous solution was cooled to 0 °C and sodium borohydride (3.1 g, 82.2 mmol, 6.0 equiv) was added in three portions over 15 min. The reaction was warmed to ambient temperature (23 °C), diluted with Et_2O (150 mL), and treated with 1N HCl (150 mL). The organic layer was separated and the aqueous layer was further extracted with Et_2O (2 x 150 mL). The organic layers were combined, washed with 1N HCl (100 mL) followed by brine (200 mL), dried with Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography (SiO_2 0 → 40% EtOAc/hexanes) provided 3.23 g (68%) cyclohexenone **2.7** as a yellow oil. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.6 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39-7.31 (m, 5H), 6.71 (s, 1H), 4.56, 4.54 (ABq, J=12 Hz, 2H), 3.45 (app m, J=6.5, 1.5 Hz, 2H), 2.74 (s, 1H), 2.53 (dt, J = 16.5, 4.5 Hz, 1H), 2.48 (m, 2H), 2.40-2.33 (m, 3H), 2.12-2.07 (m, 1H), 1.78-1.71 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 198.8, 172.1, 146.8, 138.2, 137.8, 128.2 (2), 127.5, 127.4, 126.7, 79.9, 73.0, 72.5, 37.0, 36.8, 34.1, 27.9 (3), 25.8, 25.3; IR (thin film): 3091, 3064, 3031, 2978, 1723, 1675, 1359, 1146 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{21}H_{28}LiO_4$ $[M + Li]^+$: 351.2148, found: 351.2144.



Tri-substituted cyclohexanone 2.6. Copper(I) bromide-dimethyl sulfide complex (0.6 g, 2.9 mmol, 15 mol %) was weighed into an oven-dried round-bottomed flask and suspended in THF (200 mL). HMPA (6.78 mL, 38.9 mmol, 2.0 equiv) was added and the solution became homogeneous over 10 min. Following cooling to -78 °C, 2-methyl-1-propenylmagnesium bromide (0.5 M in THF, 117 mL, 3.0 equiv) was added with vigorous stirring. A separate solution of **2.7** (6.7 g, 19.5 mmol, 1.0 equiv) in THF (10 mL) was prepared with TMSCl (6.2 mL, 48.7 mmol, 2.5 equiv) and was added to the cuprate solution *via* syringe pump over 1 h. The reaction was warmed to ambient temperature (23 °C) and stirred for 12 h. After this time, full conversion of **2.7** to the silyl enol ether had taken place, as indicated by TLC, and the reaction was subsequently diluted with Et_2O (150 mL) and quenched with saturated NH_4Cl (150 mL). Stirring was continued until the aqueous layer turned bright blue. 1N HCl (150 mL) was added and the biphasic solution was stirred vigorously until TLC indicated full conversion of the silyl enol ether to the ketone. The organic layer was separated and the aqueous layer was further extracted with Et_2O (2 x 150 mL). The organic layers were combined, washed with brine (300 mL), dried with Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography (SiO_2 0 → 30% EtOAc/hexanes) gave 5.67 g (73%) of the tri-substituted cyclohexanone **2.6** as a yellow oil. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.8 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37-7.30 (m, 5H), 4.91 (dd, *J* = 10, 1 Hz, 1H), 4.46, 4.44 (ABq, J=12, 2H), 3.50 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.26 (dd, *J* = 8.5, 7.0 Hz, 1H), 2.42 (dt, *J* = 7.0, 4.0 Hz, 2H), 2.38-2.28 (m, 4H), 2.21 (dt, *J* = 9.0, 2.0 Hz, 1H), 2.14 (dt, *J* = 13.0, 3.0 Hz, 1H), 1.89-1.82 (m, 1H), 1.76 (s, 3H), 1.71-1.64 (m, 2H), 1.57 (s, 3H), 1.45 (s, 9H) ; ^{13}C NMR (125 MHz; $CDCl_3$): δ 211.5, 172.9, 138.3, 134.0, 128.1 (2), 127.4, 127.3 (2), 126.5, 79.6, 72.9, 72.3, 53.3, 45.7, 42.4, 41.4, 33.4, 30.3, 27.9 (3), 25.6, 21.9, 18.4 ; IR (thin film): 3339, 3031, 2978, 1732, 1711, 1448, 1362 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{37}O_4$ $[M + H]^+$: 401.2692, found: 401.2683.

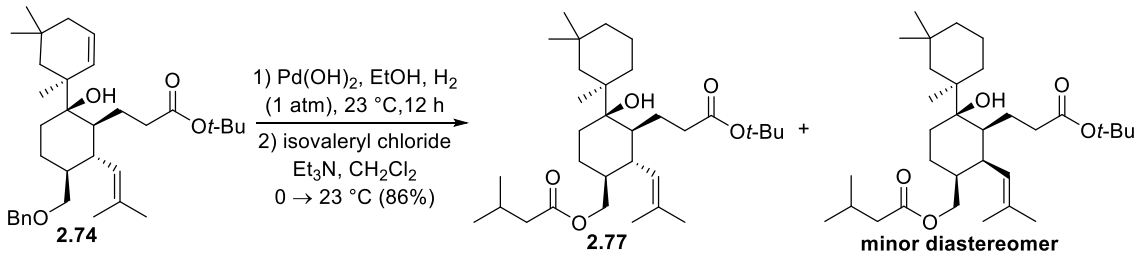


Allyl chloride 2.76. *N*-chlorosuccinimide (15.4 g, 115 mmol, 1.1 equiv) was weighed into an oven-dried round-bottomed flask and suspended in CH_2Cl_2 (200 mL). The slurry was cooled to 0 °C and Me_2S (10.0 mL, 133 mmol, 1.28 equiv) was added dropwise over 5 min giving a white solution. 3,5,5-Trimethyl-2-cyclohexen-1-ol (16.0 mL, 105 mmol, 1.0 equiv) was added dropwise with vigorous stirring to provide a clear solution. Within 5 min, precipitate formed. The reaction proceeded at 0 °C for 2 h, was concentrated *in vacuo*, taken up in pentane (250 mL), immediately forming a white precipitate, and placed in the freezer for 4 h. Once the precipitation was complete, the mixture was washed with brine (2 x 150 mL), dried with Na_2SO_4, and concentrated *in vacuo*. Allyl chloride **2.76** was isolated as a yellow oil (14.36 g, 79%) that was taken on without purification. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.8 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.51-5.5 (m, 1H), 4.66-4.63 (m, 1H), 2.0-1.94 (m, 2H), 1.72 (s, 3H), 1.69-1.66 (m, 2H), 1.04 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 137.3, 122.0, 56.7, 45.7, 43.5, 31.7, 30.7, 25.8, 23.6; IR (thin film): 3044, 2951, 2898, 1667, 1460, 1436, 817, 726 cm^{-1}. The parent molecular ion (M+) was unable to be detected by various methods.



Homoallylic alcohol 2.74. To an oven-dried round-bottomed flask, zinc (9.0 g, 136 mmol, 3.6 equiv) and LiCl (1.16 g, 27.3 mmol, 0.7 equiv) were added. The flask and its contents were then

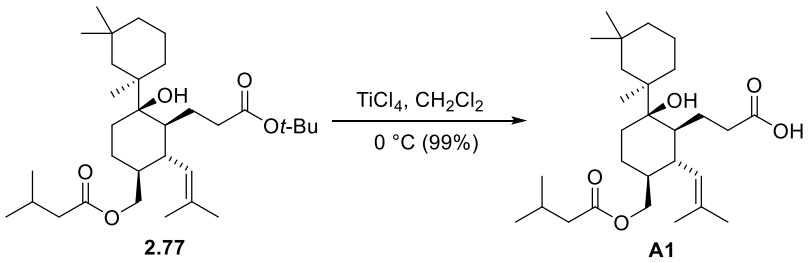
flame-dried under vacuum. After cooling for 3 min, THF (13.7 mL) was slowly added with vigorous stirring. Fast addition of 1,2-dibromoethane (0.71 mL, 6.8 mmol, 0.18 equiv) lead to exothermic activation of the zinc, observed by rapid evolution of ethene gas. Note: if this process does not occur on its own accord, a heat gun may be employed to initiate the activation. Excess pressure was eliminated by vacuum. The solution was cooled slightly at 23 °C over 5 min before being further cooled to 0 °C. A solution of the **2.6** (0.2 g, 0.5 mmol, 0.013 equiv) in THF (2.4 mL) was added dropwise by syringe followed by slow addition of **2.76** (6 g, 37.9 mmol, 1 equiv) in THF (9.6 mL) *via* syringe pump at a rate of 4.5 mL/h. The reaction proceeded for an additional 18 h at 0 °C before being quenched with saturated NH_4Cl solution (100 mL). The organic phase was separated and the aqueous phase was further extracted with EtOAc (3 x 100 mL). The organic layers were combined, washed with brine (200 mL), dried with Na_2SO_4, and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2 0 → 50% EtOAc/hexanes) yielded 93 mg (35%) of the desired homoallylic alcohol **2.74** as a pale yellow oil. TLC (EtOAc:hexanes, 2:8 *v*/*v*): R_f = 0.6 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36-7.31 (m, 5H), 5.93 (dd, J=10.5, 2.5 Hz, 1H), 5.57 (ddd, J=10.5, 6.5, 2 Hz, 1H), 4.74 (ddd, J=10.5, 1, 1 Hz, 1H), 4.46, 4.43 (ABq, J=12 Hz, 2H), 3.43 (dd, J=9, 3.5 Hz, 1H), 3.17 (dd, J=9, 7.5 Hz, 1H), 2.36 (m, 1H), 2.26 (q, J=10.5 Hz, 1H), 2.01 (m, 1H), 1.83 (m, 6H), 1.71 (s, 3H), 1.62 (s, 3H), 1.45 (m, 3H), 1.42 (s, 9H), 1.28 (m, 4H), 1.15 (s, 3H), 0.99 (s, 6H) ; ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.4, 138.8, 133.9, 132.3, 128.2 (2), 128.1, 127.5 (2), 127.3, 123.0, 79.4, 79.3, 73.8, 73.0, 45.0, 44.3, 42.6. 41.1, 39.8, 38.1, 33.9, 33.8, 32.5, 30.5, 28.5, 28.2 (3), 25.8, 25.3, 24.6, 24.3, 18.7; IR (thin film): 3517, 3028, 2928, 1729, 1454, 1365 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{34}H_{53}O_4$ $[M + H]^+$: 525.3944, found: 525.3949.



Isovaleric ester 2.77. To a solution of **2.74** (0.149 g, 0.284 mmol, 1.0 equiv) in EtOH (16 mL) was added $Pd(OH)_2$ on carbon (0.16 g, 20 wt%, 0.228 mmol,). A H_2 balloon was placed atop the flask and the flask was evacuated three times under high vacuum. The reaction proceeded for 18 h at which point an aliquot was removed to check the reaction with ^{1}H NMR to insure the cyclohexene had been reduced. The reaction mixture was filtered through a pad of Celite (washed with EtOAc), concentrated *in vacuo*, and taken on directly to the next step.

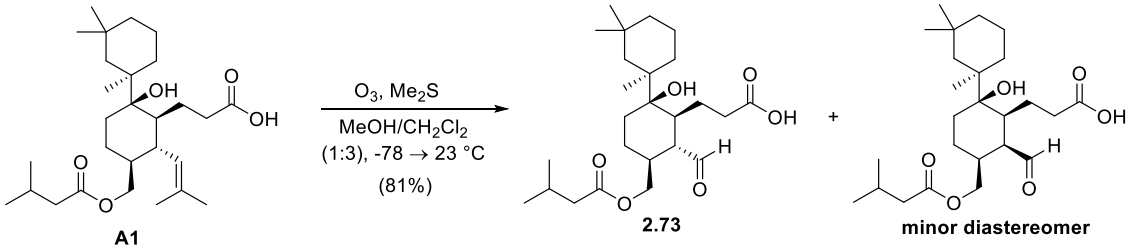
The crude primary alcohol was dissolved in CH_2Cl_2 (10 mL) and Et_3N (0.24 mL, 1.94 mmol, 6.8 equiv) and cooled to 0 °C. Isovaleryl chloride (0.15 mL, 1.39 mmol, 4.3 equiv) was added dropwise and the reaction proceeded for 5 h at which point TLC showed completion of the reaction. The crude reaction mixture was concentrated directly *in vacuo* and purified by column chromatography (SiO_2 0 → 30% EtOAc/hexanes) to give 0.127 g (86%) of **2.77**. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.75 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.72 (d, J = 10.5 Hz, 1H), 4.03 (dd, J = 10.5, 2.0 Hz, 1H), 3.74 (dd, J = 10.0, 7.5 Hz, 1H), 2.44-2.39 (m, 1H), 2.30-2.27 (m, 1H), 2.15 (dd, J = 6.5, 1.5 Hz, 2H), 2.11-2.04 (m, 3H), 1.91 (dt, J = 14.0, 3.5 Hz, 2H), 1.70 (s, 3H), 1.62 (s, 3H), 1.54-1.48 (m, 4H), 1.40 (s, 9H), 1.27-1.19 (m, 10H), 1.03 (s, 3H), 0.99 (s, 3H), 0.94 (d, J = 1.5, 3H), 0.93 (d, J = 1.5, 3H), 0.89 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.4, 173.1, 132.9, 127.4, 79.3, 78.6, 75.1, 67.4, 45.0, 44.3, 43.4, 42.6, 41.2, 40.7, 38.4, 36.0, 33.2, 32.2, 32.1, 30.9, 28.0 (3), 27.4, 26.1, 25.7, 25.5, 24.4, 22.4, 20.9, 19.3, 18.6; IR (thin film):

3532, 2960, 2922, 2869, 1729, 1708, 1463 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{32}H_{56}LiO_5$ [M + Li]$^+$: 527.4288, found: 527.4310.

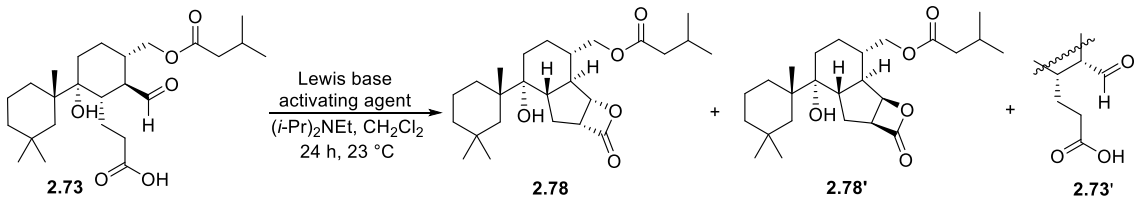


2.77 A1

Carboxylic acid A1. A solution of isovaleric ester **2.77** (0.085 g, 0.16 mmol, 1.0 equiv) in CH_2Cl_2 (4.0 mL) was cooled to 0 °C. Titanium tetrachloride (1.0 M in CH_2Cl_2, 0.41 mL, 2.5 equiv) was added dropwise *via* syringe. After 10 min, TLC indicated reaction completion and quenching was done at 0 °C with saturated $NaHCO_3$ (15 mL). After stirring for 2 min, 1N HCl (10 mL) was added. The organic phase was separated and the aqueous layer was further extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were combined, washed with brine (15 mL), dried with Na_2SO_4, and concentrated *in vacuo* to give 73.5 mg of carboxylic acid **A1** (99%.). The compound was of sufficient purity to be taken on to the next step without further purification. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.7 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.74-4.72 (d, 10 Hz, 1H), 4.05 (dd, J = 11.0, 6.5, 1H), 3.75 (dd, J = 11.0, 7.5 Hz, 1H), 2.62 (ddd, J=16, 12.5, 8.5 Hz, 1H), 2.34-2.22 (m, 2H), 2.17 (app d, J = 6.5 Hz, 2H), 2.11-2.06 (m, 1H), 2.02-1.95 (m, 1H), 1.71 (s, 3H), 1.62 (s, 3H), 1.56-1.50 (m, 3H), 1.46-1.29 (m, 12H), 1.24-1.16 (m, 2H), 1.04 (s, 3H), 0.99 (s, 3H), 0.95 (d, J = 0.8, 3H), 0.94 (d, J = 0.8, 3H), 0.88 (s, 3H), O-H proton not observed; ^{13}C NMR (125 MHz; $CDCl_3$): δ 180.1, 173.3, 133.3, 127.2, 78.8, 67.4, 44.9, 44.3, 43.5, 42.6, 41.3, 40.4, 38.5, 36.0, 32.3, 32.2, 31.4, 31.0, 27.5, 25.8, 25.7, 25.6, 24.5, 22.4 (2), 21.0, 19.4, 18.6; IR (thin film): 3339, 3031, 2978, 1732, 1711, 1448, 1362 cm^{-1}; HRMS (-ESI) *m*/*z* calcd for $C_{28}H_{47}O_5$ [M - H]$^-$: 463.3424, found: 463.3429.



Aldehyde acid 2.73. A solution of carboxylic acid **A1** (0.151 g, 0.325 mmol, 1.0 equiv) was dissolved in a mixed solvent system of MeOH/CH_2Cl_2 (1:5, 6 mL: 30 mL) and cooled to -78 °C. Ozone was bubbled through the reaction solution until a blue color persisted. Excess ozone was removed by blowing N_2 gas over the solution with stirring for 10 min. DMS (0.24 mL, 32.5 mmol, 10.0 equiv) was added and the reaction was slowly warmed to ambient temperature (23 °C) over 3 h at which time TLC indicated the reaction was complete. Purification by flash chromatography (SiO_2 0 → 60% EtOAc/hexanes) gave 0.116 g (81%) of aldehyde acid **2.73** as a colorless oil. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$): 9.57 (dd, J=4, 1 Hz, 1H), 3.96-3.88 (qd, J=11.5, 6.5 Hz, 2H), 2.74 (ddd, J=16.0, 12.5, 3.5 Hz, 1 H), 2.51 (m, 2H), 2.44 (app d, J=5 Hz, 2H), 2.11-2.04 (m, 3H), 1.72-1.54 (m, 8H), 1.48-1.25 (m, 8H), 1.1 (s, 3H), 1.0 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.9 (s, 3H), O-H proton not observed ; ^{13}C NMR (125 MHz; $CDCl_3$): δ 204.0, 179.1, 172.8, 78.3, 66.8, 54.1, 44.0, 43.2, 42.6, 39.3, 38.4, 36.2, 35.9, 32.3, 31.8, 30.9, 29.6, 27.4, 26.0, 25.5, 23.0, 22.4, 22.3, 20.7, 19.2 ; IR (thin film): 3529, 2951, 2928, 2869, 1726, 1709 cm^{-1}; HRMS (-ESI) *m*/*z* calcd for $C_{25}H_{41}O_6$ [M - H]$^-$: 437.2909, found: 437.2926.



Representative procedure for Nucleophile Catalyzed Aldol-Lactonization process as described for β-lactone 13.

Racemic:

Entry 1. To an oven-dried round-bottomed flask, modified Mukaiyama's reagent (7.5 mg, 213 μmol, 3.0 equiv) was added and azeotroiped with xylenes *in vacuo*. It was then dissolved in in CH_2Cl_2 (1.5 mL) and Et_3N (50.0 μL, 355 μmol, 5.0 equiv). A separate solution of **2.73** (31.1 mg, 71 μmol, 1.0 equiv) was prepared in CH_2Cl_2 (0.5 mL) and was subsequently added *via* syringe pump over 1 h. Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (3:1); **2.78** (30%, 9.1 mg) and **2.78'** (9.4%, 2.8 mg).

Entry 2. Prepared according to representative procedure (**Entry 1**) utilizing TsCl (3.5 mg, 18 μmol, 1.5 equiv) and (±)-HBTM (3.3 mg, 12.3 μmol, 1.0 equiv) in CH_2Cl_2 (0.27 mL), (*i*-Pr)$_2$NEt (10 μL, 6.16 μmol, 5.0 equiv), **2.73** (5.4 mg, 12.3 μmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL). Both TsCl and (±)-HBTM were azeotroped with xylenes *in vacuo*. Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (1:1); **2.78** (10%, 500 μg) and **2.78'** (10%, 500 μg).

Asymmetric:

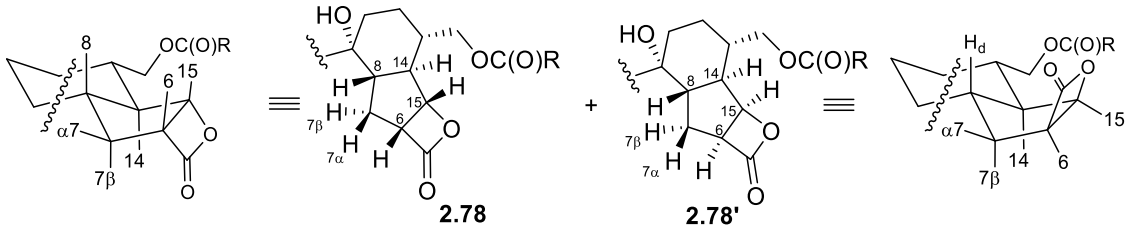
Entry 3. To an oven-dried round-bottomed flask, modified Mukaiyama's reagent (43 mg, 123 μmol, 3.0 equiv) was added followed by (*S*)-HBTM (11 mg, 41 μmol, 1.0 equiv). The two solids were azeotroped with xylenes *in vacuo*. The mixture was dissolved in CH_2Cl_2 (1.0 mL) and (*i*-Pr)$_2$NEt (34 μL, 205 μmol, 5.0 equiv) with stirring. A separate solution of **2.73** (18 mg, 4.1 μmol, 1.0 equiv) was prepared in CH_2Cl_2 (2 mL) and was subsequently added *via* syringe pump over 1 h. The reaction then proceeded for an additional 18 h, was concentrated *in vacuo*,

and purified (SiO_2 0 → 30% EtOAc/hexanes) to provide a mixture of diastereomers (1:1): **(-)-2.78** (2.7 mg,16%) and **(-)2.78'** (2.8 mg, 16%). TLC (EtOAc:hexanes, 3:7 *v*/*v*).

β-lactone **(-)-13**. R_f = 0.8; $[\alpha]_D^{20}$ = -36.00 (*c* = 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): 4.76 (dd, J=6.0, 4.5 Hz, 1H), 4.15 (dd, J=11.5, 6.5, 1H), 4.04 (dd, J=11.0, 6.0, 1H), 3.92 (dt, J= 10, 6 Hz, 1H), 2.22 (dd, J=7.5, 2 Hz, 2H), 2.15-2.09 (m, 3H), 2.03-1.99 (m, 3H), 1.76-1.71 (m, 3H), 1.41-1.37 (m, 7H), 1.28-1.27 (m, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H) ; ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.1, 172.2, 78.8, 77.0, 67.5, 54.4, 53.6, 48.6, 44.2, 43.3, 41.7, 39.5, 38.6, 36.1, 31.3, 30.8, 29.7, 27.5, 26.9, 25.6, 25.2, 22.4, 21.1, 19.0 (2); IR (thin film): 2957, 2860, 1824, 1735, 1637, 1297 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{40}LiO_5$ $[M + Li]^+$: 427.3036, found: 427.3031.

β-lactone **(-)-2.78'**. R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$): 4.76 (dd, J=6.0, 4.5 Hz, 1H), 4.15 (dd, J=11.5, 6.5 Hz, 1H), 4.04 (dd, J=11.0, 6.0 Hz, 1H), 3.92 (dt, J= 10, 6 Hz, 1H), 2.22 (dd, J=7.5, 2 Hz, 2H), 2.15-2.09 (m, 3H), 2.03-1.99 (m, 3H), 1.76-1.71 (m, 3H), 1.41-1.37 (m, 7H), 1.28-1.27 (m, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H) ; ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.1, 172.2, 78.8, 77.0, 67.5, 54.4, 53.6, 48.6, 44.2, 43.3, 41.7, 39.5, 38.6, 36.1, 31.3, 30.8, 29.7, 27.5, 26.9, 25.6, 25.2, 22.4, 21.1, 19.0 (2); IR (thin film): 2957, 2860, 1824, 1735, 1637, 1297 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{40}LiO_5$ $[M + Li]^+$: 427.3036, found: 427.3031.

Table A1. Analysis of coupling constants for β-lactones **2.78** and **2.78'**.



Cmpd.	$J_{14,15}$ (Hz)	$J_{15,6}$ (Hz)	$J_{6,7\alpha}$ (Hz)	$J_{6,7\beta}$ (Hz)	$J_{7\alpha,8}$ (Hz)	$J_{7\beta,8}$ (Hz)	$J_{8,14}$ (Hz)	$J_{13,14}$ (Hz)
2.78	4.5	6.0	6.0	11.0	nd	nd	nd	nd
2.78'	4.0	4.0	4.0	8.0	5.5	nd	11.5	nd

*Note: Indistinguishable couplings, due to aliphatic overlap are indicated by 'nd' in table.

Epimerized aldehyde-acid **(±)-2.73'**. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.28; ^{1}H NMR (500 MHz, $CDCl_3$): 9.58 (d, J=2.5 Hz, 1H), 4.01 (dd, J=11, 5.5 Hz, 1H), 3.94 (dd, J=11, 5.5 Hz, 1H), 2.81-2.75 (m, 1H), 2.65-2.61 (m, 2H), 2.55-2.48 (m, 1H), 2.18 (app d, 2H), 2.14-2.01 (m, 5H), 1.74-1.64 (m, 6H), 1.44-1.34 (m, 6H), 1.09 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 204.1, 179.1, 172.8, 78.3, 66.8, 54.1, 44.0, 43.2, 42.7, 39.3, 38.4, 36.2, 35.9, 32.3, 31.8, 30.9, 29.6, 27.4, 26.0, 25.5, 23.0, 22.4 (2), 20.7, 19.2.

Entry 4. Prepared according to representative procedure (**Entry 3**) utilizing modified Mukaiyama's reagent (24 mg, 68 µmol, 3.0 equiv) and (*R*)-HBTM (6.1 mg, 23 µmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL), (*i*-Pr)$_2$NEt (19 µL, 114 µmol, 5.0 equiv), **2.73** (10 mg, 2.3 µmol, 1.0 equiv) in CH_2Cl_2 (2 mL). Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (1:2): **(+)-2.78** (6.3%, 600 µg) and **(+)-2.78'** (12.5%, 1.2 mg).

Entry 5. Prepared according to representative procedure (**Entry 3**) utilizing modified Mukaiyama's reagent (15 mg, 42 µmol, 3.0 equiv) and (-)-BTM (3.5 mg, 13.9 µmol, 1.0 equiv) in CH_2Cl_2 (0.3 mL), $(i\text{-Pr})_2NEt$ (11 µL, 69 µmol, 5.0 equiv), **2.73** (6.1 mg, 13.9 µmol, 1.0 equiv) in CH_2Cl_2 (1.0 mL). Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (1.2:1): **(+)-2.78** (11.4%, 600 µg) and **(+)-2.78'** (9.5%, 500 µg).

Entry 6. Prepared according to representative procedure (**Entry 3**) utilizing modified Mukaiyama's reagent (12 mg, 34 µmol, 3.0 equiv) and TMSQD (4.5 mg, 11.4 µmol, 1.0 equiv) in CH_2Cl_2 (0.25 mL), $(i\text{-Pr})_2NEt$ (10 µL, 57 µmol, 5.0 equiv), **2.73** (5.0 mg, 11.4 µmol, 1.0 equiv) in CH_2Cl_2 (1.0 mL). Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (1.3:1): **(-)-2.78** (19%, 900 µg) and **(-)-2.78'** (13.9%, 666 µg) and **(± 2.73')** (27%, 1.35 mg).

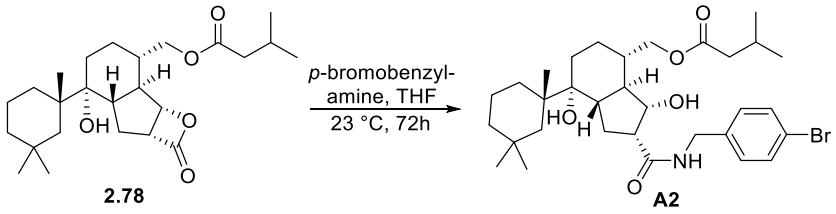
Entry 7. Prepared according to representative procedure (**Entry 3**) utilizing modified Mukaiyama's reagent (24 mg, 68 µmol, 3.0 equiv) and TMSQN (9.0 mg, 23.0 µmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL), $(i\text{-Pr})_2NEt$ (18.7 µL, 114 µmol, 5.0 equiv), **2.73** (10.0 mg, 23.0 µmol, 1.0 equiv) in CH_2Cl_2 (2.0 mL). Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (2:1): **(+)-2.78** (10 %, 900 µg), $[\alpha]_D^{20}$ = +10.77 (*c* = 0.09, $CHCl_3$), **(+)-2.78'** (4%, 39 µg) and **(±)-2.73'** (34%, 3.4 mg).

Entry 8. Prepared according to representative procedure (**Entry 3**) utilizing modified Mukaiyama's reagent (12 mg, 34 µmol, 3.0 equiv) and TMSQN (4.5 mg, 11.4 µmol, 1.0 equiv) in CH_2Cl_2 (0.25 mL), $(i\text{-Pr})_2NEt$ (3.7 µL, 23 µmol, 2.0 equiv), **2.73** (5.0 mg, 11.4 µmol, 1.0 equiv) in CH_2Cl_2 (1.0 mL). Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of

diastereomers (5:1): **(+)-2.78** (12.5%, 600 µg) and **(-)-2.78'** (2.7%, 130 µg) and **(±)-2.73'** (18%, 900 µg).

Entry 9. Prepared according to representative procedure (**Entry 3**) utilizing modified Mukaiyama's reagent (24 mg, 68 µmol, 3.0 equiv) and TMSQN (2.4 mg, 6.2 µmol, 0.27 equiv) in CH_2Cl_2 (0.5 mL), (i-Pr)$_2$NEt (18.7 µL, 114 µmol, 5.0 equiv), **2.73** (10.0 mg, 23.0 µmol, 1.0 equiv) in CH_2Cl_2 (2.0 mL). Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a mixture of diastereomers (2:1): **(+)-2.78** (12% on 5 mg scale, 600 µg and 6.2% on 10 mg scale, 600 µg) and **(+)-2.78'** (3%, 300 µg). and **(±)-2.73** (25%, 2.5 mg).

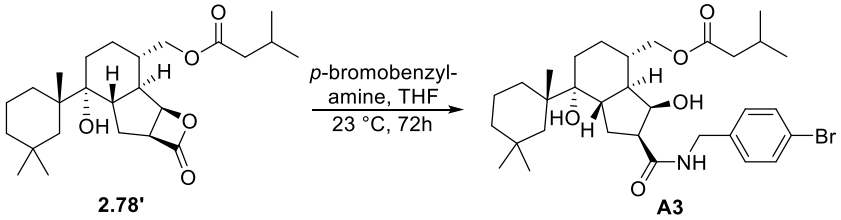
***p*-Bromobenzyl amide.**



2.78 A2

From entry 3: **(-)-A2.** To a vial containing *p*-bromobenzylamine (0.9 mg, 4.8 µmol, 1.8 equiv), a separately prepared solution of **(-)-2.78** (1.1 mg, 2.6 µmol, 1.0 equiv) in THF (0.2 mL) was added. The reaction proceeded for 72 hours after which it was loaded directly onto one 10 cm x 20 cm TLC plate and eluted three times in 30:70 EtOAc/hexanes, *v*/*v*, R_f = 0.2. The silica was washed with CH_2Cl_2 and provided (-)-**A2** (600 µg, 31%) upon concentration; (Note: A sufficient amount of (-)-**S2** was isolated to obtain ^{1}H NMR but was insufficient for ^{13}C NMR) ^{1}H NMR (500 MHz, $CDCl_3$): 7.48 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 6.09 (app t, J = 5 Hz, 1H), 4.50 (dd, J = 15, 6 Hz, 1H), 4.42-4.36 (m, 2H), 4.17-4.12 (m, 2H), 4.00 (app t, J = 9 Hz, 1H), 2.82 (q, J = 8.0 Hz, 2H), 2.22 (app d, J = 7.5 Hz, 2H), 2.16-2.06 (m, 4H), 2.02-1.96 (m, 2H),

1.83-1.71 (m, 6H), 1.38-1.36 (m, 3H), 1.30-1.27 (m, 3H), 1.02 (s, 3H), 1.00 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H); IR (thin film): 3100, 2925, 1726, 1643, 1457, 1256, 1157, 1066 cm^{-1}; HRMS (ESI+) *m*/*z* calcd. For $C_{32}H_{49}BrNO_5$ $[M+H]^+$: 606.2794; found 606.2773. Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.56 min, t_{minor} = 10.2 min; 78% *ee*.



From Entry 3: **(-)-A3**. To a vial containing *p*-bromobenzylamine (1.7 mg, 9.1 µmol, 2.75 equiv), a separately prepared solution of **(-)-2.78'** (1.2 mg, 2.8 µmol, 1.0 equiv) in THF (0.2 mL) was added. The reaction proceeded for 72 hours after which it was loaded directly onto one 10 cm x 20 cm TLC plate and eluted three times in 30:70 EtOAc/hexanes, *v*/*v*, R_f = 0.2. The silica was washed with CH_2Cl_2 and provided (-)-**A3** (1.3 mg, 75%) upon concentration; (Note: A sufficient amount of (-)-**A3** was isolated to obtain ^{1}H NMR but was insufficient for ^{13}C NMR) ^{1}H NMR (500 MHz, $CDCl_3$): 7.49 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 6.28-6.26 (m, 1H), 4.53 (dd, *J* = 15.5, 6.5 Hz, 1H), 4.42-4.37 (m, 3H), 4.17 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.02 (dd, *J* = 11.0, 6.5 Hz, 1H), 2.67-2.63 (m, 1H), 2.36-2.29 (m, 1H), 2.21 (app d, *J* = 6.5 Hz, 2H), 2.15-2.01 (m, 4H), 1.88-1.81 (m, 1H), 1.76-1.71 (m, 3H), 1.42-1.35 (m, 5H), 1.25-1.17 (m, 5H), 1.07 (s, 3H), 1.02 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H); IR (thin film): 3100, 2925, 1726, 1643, 1457, 1256, 1157, 1066 cm^{-1}; HRMS (ESI+) *m*/*z* calcd. For $C_{32}H_{49}BrNO_5$ $[M+H]^+$: 606.2794; found 606.2773. **(-)-A3**: Enantiomeric excess was determined by HPLC analysis in comparison

with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 90:10, flow rate 0.9 mL/min, λ = 230 nm: t_{major} = 14.218 min, t_{minor} = 35.013 min; 20% *ee*.

*For remaining entries, the procedure followed protocols for Entry 1. Yields for **A2** and **A3** were not determined, and, upon preparatory TLC, the samples were immediately subjected to HPLC analysis for ee determination.*

Entry 4. (+)-A2: Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.62 min, t_{minor} = 10.3 min; 84% *ee*; **(+)-A2**: Chiralcel IA column: hexanes:*i*PrOH = 90:10, flow rate 0.9 mL/min, λ = 230 nm: t_{major} = 14.218 min, t_{minor} = 35.013 min; 20% *ee*.

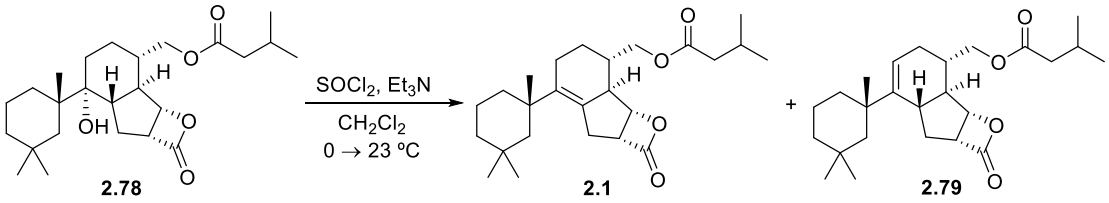
Entry 5. (+)-A2: Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.57 min, t_{minor} = 10.32 min; 81% *ee*; **(+)-A2**: Chiralcel IA column: hexanes:*i*PrOH = 90:10, flow rate 0.9 mL/min, λ = 230 nm: t_{major} = 14.218 min, t_{minor} = 35.013 min; 60% *ee*.

Entry 6. (-)-A2: Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.37 min, t_{minor} = 10.21 min; 93% *ee*; **(-)-A2**: Chiralcel IA column: hexanes:*i*PrOH = 90:10, flow rate 0.9 mL/min, λ = 230 nm: t_{major} = 14.218 min, t_{minor} = 35.013 min; 77% *ee*.

Entry 7. (+)-A2: Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.57 min, t_{minor} = 10.32 min; 90% *ee*.

Entry 8. (+)-A2: Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.57 min, t_{minor} = 10.32 min; 92% *ee*.

Entry 9. (+)-A2: Enantiomeric excess was determined by HPLC analysis in comparison with authentic racemic material using a Chiralcel IA column: hexanes:*i*PrOH = 75:25, flow rate 1.0 mL/min, λ = 210 nm: t_{major} = 8.57 min, t_{minor} = 10.32 min; 90% *ee*.



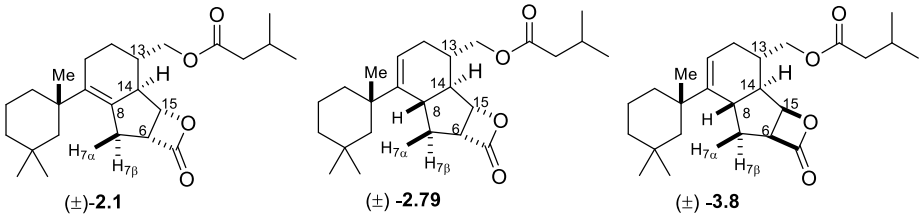
(+)-Spongiolactone, 2.1 and Regioisomeric (+)-Spongiolactone 2.79. β-lactone **(+)-2.78** (2.7 mg, 6.4 μmol, 1.0 equiv) was dissolved in CH_2Cl_2 and cooled to 0 °C. $SOCl_2$ (1.99 μL, 27.3 μmol, 4.25 equiv) was added dropwise over 2 min. The reaction was warmed to ambient temperature (23 °C) and stirred until TLC indicated its completion. The reaction was concentrated *in vacuo* and loaded directly onto a silica column for purification (SiO_2 0 → 30% EtOAc/hexanes) providing 1.4 mg of **2.1** (54%) as well as 600 μg of regioisomeric olefin **2.79** (23%).

Spongiolactone (**2.1**): TLC (EtOAc:hexanes, 2:8 *v*/*v*): R_f = 0.8; $[\alpha]_D^{20}$ = +45.5 (*c* = 0.04, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): 4.73 (dd, *J* = 3.7, 3.7 Hz, 1H), 4.14, 4.04 (dd *J* = 11.1, 6.2 Hz, 2H),

3.90 (dt, *J* = 11.2, 5.7 Hz, 1H), 3.05 (dd, *J* = 10.5, 5.5 Hz, 1H), 2.67 (d, *J* = 13.0 Hz, 1H), 2.43 (d, *J* = 11.0 Hz, 1H), 2.29 (d, *J* = 17.0 Hz, 1H), 2.20 (app d, *J* = 7.1 Hz, 2H), 2.11-2.06 (m, 2H),1.93 (m, 1H), 1.83 (m, 1H), 1.71-1.65 (m, 2H), 1.48-1.44 (m, 1H), 1.31-1.24 (m, 4H), 1.12-1.11 (m, 2H), 0.97 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.85 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.9, 171.8, 139.4, 133.7, 79.9, 67.6, 54.3, 50.2, 49.9, 43.3, 39.7, 39.4, 38.3, 36.8, 32.7, 31.4, 30.5, 30.3, 26.8, 26.5, 26.3, 25.6, 22.4 (2), 20.9 ; IR (thin film): 2951, 2925, 2866, 1829, 1725, 1626, 1462, 1294, 1107 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{38}LiO_4$ [M + Li]$^+$: 409.2925, found: 409.2938.

Regioisomeric spongiolactone (**2.79**): TLC (EtOAc:hexanes, 2:8 *v*/*v*): R_f = 0.7 ; $[\alpha]_D^{20}$ = +33.7 (*c* = 0.03, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): 5.58 (m, 1H), 4.84 (app t, *J* = 5.5 Hz, 1H), 4.15 (dd, *J* = 5.5, 2.0 Hz, 2H), 4.07-4.02 (m, 1H), 2.55-2.50 (m, 1H), 2.49-2.43 (m, 1H), 2.34-2.30 (m, 1H), 2.25 (app d, *J* = 6.5 Hz, 2H), 2.17-2.10 (m, 2H), 2.08-2.03 (m, 2H), 2.00-1.95 (m, 2H), 1.89-1.84 (m, 2H), 1.82-1.78 (m, 1H), 1.21-1.18 (m, 4H), 1.04 (s, 3H), 1.00 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.1, 172.1,145.3, 120.0, 77.1, 67.1, 55.3, 52.4, 49.2, 48.9, 43.3, 39.8, 39.5, 37.8, 36.0, 32.0, 31.4, 30.74, 30.72, 29.7, 25.6, 22.4 (2), 19.9, 14.1; IR (thin film): 2951, 2925, 2866, 1829, 1725, 1626, 1462, 1294, 1107 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{38}LiO_4$ [M + Li]$^+$: 409.2925, found: 409.2938.

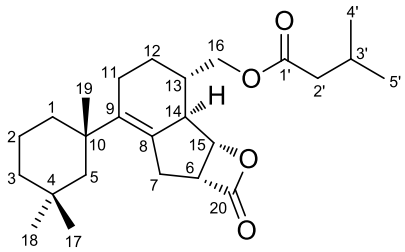
Table A2. Analysis of coupling constants for spongiolactone ((±)-**2.1**), regio-spongiolactone ((±)-**2.79**), and regio, bis-epi spongiolactone ((±)-**3.8**).



Cmpd	$J_{14, 15}$ (Hz)	$J_{15, 6}$ (Hz)	$J_{6, 7\alpha}$ (Hz)	$J_{6, 7\beta}$ (Hz)	$J_{7\alpha, 8}$ (Hz)	$J_{7\beta, 8}$ (Hz)	$J_{8, 14}$ (Hz)	$J_{13, 14}$ (Hz)
(±)-**3**	3.5	6.0	6.0	11.0	NA	NA	NA	11.0
(±)-**16**	6.0	6.0	4.0	10.5	5.0	1.5	11.5	nd
(±)-**17**	3.5	3.5	7.5	4.0	5.5	nd	12.5	nd

*Note: Indistinguishable couplings, due to aliphatic overlap are indicated by 'nd' in table. NA denotes the lack of proton 8 in spongiolactone ((±)-**2.1**).

Table A3. Comparison of ^{1}H NMR data of natural[1] and synthetic spongiolactone and 3'-norspongiolactone[2] in $CDCl_3$.



1.95-1.83 (m, 2H), 1.74-1.64 (m, 2H), 1.52-1.39 (m, 1H), 1.34-1.22 (m, 4H), 1.13-1.07 (m, 2H), 0.95 (d, 6H), 0.94 (s, 3H), 0.85 (s, 3H), 0.78 (s, 3H);

Pos.	**^{1}H NMR data-natural spongiolactone (500 MHz)**	**^{1}H NMR data-synthetic spongiolactone (500 MHz)**	**Δ ppm (natural-synthetic)**	**^{1}H NMR data-3'-norspongiolactone (400 MHz)**
1	1.90	1.95-1.83 (m, 2H)	-	1.80 (m)
2	1.47[i], 1.10	1.46, 1.11 (dt, J = 11.5, 4.5 Hz)	0.01, 0.01	1.45 (m), 1.08 (m)
5	1.93, 1.02	1.93 (m), 1.02	0.0, 0.0	1.90 (m), 1.03 (m)

[i] J values were not reported in the original isolation paper[1] for these resonances.

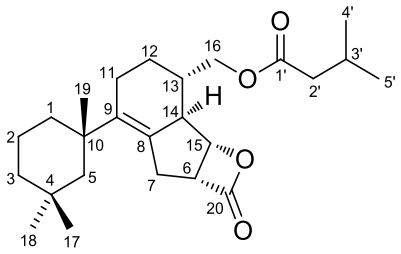
[1]. L. Mayol, V. Piccialli, D. Sica, *Tetrahedron Lett.* **1987**, *28*, 3601-3604.
[2]. M. E. Rateb, W. E. Houssen, M. Schumacher, W. T. Harrison, M. Diederich, R. Ebel, M. Jaspars, *J. Nat. Prod.* **2009**, *72*, 1471-1476.

Table A3 Continued.

Pos.	^{1}H NMR data-natural spongiolactone (500 MHz)	^{1}H NMR data-synthetic spongiolactone (500 MHz)	Δ ppm (natural-synthetic)	^{1}H NMR data-3'-norspongiolactone (400 MHz)
6	3.90 (ddd, *J* = 11.0, 6.0, 6.0 Hz)	3.90 (overlapping ddd, *J* = 11.0, 6.0, 6.0 Hz)	0.0	3.88 (ddd, *J* = 16.0, 11.2, 6.0 Hz)
7	3.04[a], 2.66	3.05 (dd, *J* = 13.5, 10.5 Hz), 2.67 (app br d, *J* = 12.5 Hz, 1H)	0.01, 0.01	3.02, (dd, *J* = 16.0, 11.0 Hz), 2.68, (dd, *J* = 16.0, 6.0 Hz)
11	2.29	2.28 (app br d, *J* = 17.0 Hz, 1H)	0.01	2.28 (m)
13	1.66	1.67	0.01	1.63 (m)
14	2.42	2.42 (app br d, *J* = 8.5 Hz, 1H)	0	2.39 (d, *J* = 11 Hz)
15	4.73 (dd, *J* = 6.0, 4.4 Hz)	4.73 (dd, *J* = 6.0, 3.5 Hz)	0.0	4.71 (dd, *J* = 6.0, 3.8 Hz)
16	4.14 (dd, *J* = 11.0, 6.6 Hz), 4.04 (dd, *J* = 11.0, 6.6 Hz)	4.14 (dd, *J* = 11.0, 6.0 Hz), 4.04 (dd, *J* = 11.0, 6.0 Hz)	0.0, 0.0	4.12, 4.02 (dd, *J* = 11.2, 6.5 Hz)
17	0.77	0.78 (s)	0.01	0.76 (s)
18	0.84	0.85 (s)	0.01	0.83 (s)
19	0.96	0.97 (s)	0.01	0.95 (s)
2'	2.19[i]	2.21-2.19 (m)	-	-
3'	2.07[i]	2.11-2.05 (m)	-	-
4', 5'	0.94	0.94 (d)	0.0	-

Note: Chemical shifts and corresponding *J* values are not for protons on carbons 3, 4, and 12 due to overlap of aliphatic protons.

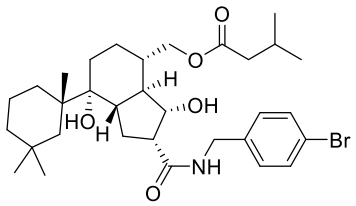
Table A4. Comparison of ^{13}C NMR data of natural[1] and synthetic spongiolactone and 3'-norspongiolactone[2] in $CDCl_3$.



Pos.	^{13}C NMR data-Natural (125 MHz)	^{13}C NMR data-Synthetic (125 MHz)	Δ ppm (natural-synthetic)	^{13}C NMR data-norspongiolactone (100 MHz)
1	38.3	38.3	0	38.3
2	20.9	20.9	0	20.9
3	39.7	39.6	0.1	39.6
4	31.4	31.4	0	31.4
5	49.9	49.8	0.1	49.9
6	54.3	54.1	0.2	54.1
7	30.5	30.6	0.1	30.4
8	133.7	133.5	0.2	133.5
9	139.4	138.9	0.5	139.0
10		31.4		31.4
11	26.3	26.0	0.3	26.2
12	26.8	26.7	0.1	26.6
13	36.8	36.6	0.2	36.6
14	50.2	50.1	0.1	50.2
15	79.9	80.0	0.1	80.1
16	67.6	67.7	0.1	67.8
17	26.5	26.2	0.3	26.0
18	32.7	33.0	0.3	32.9
19	30.3	30.4	0.1	30.4
20	171.8	172.2	0.4	172.2
1'	172.9	173.2	0.3	173.7
2'	43.3	43.3	0	-
3'	25.6	25.6	0	-
4'	22.4	22.4	0	-
5'	22.4	22.4	0	-

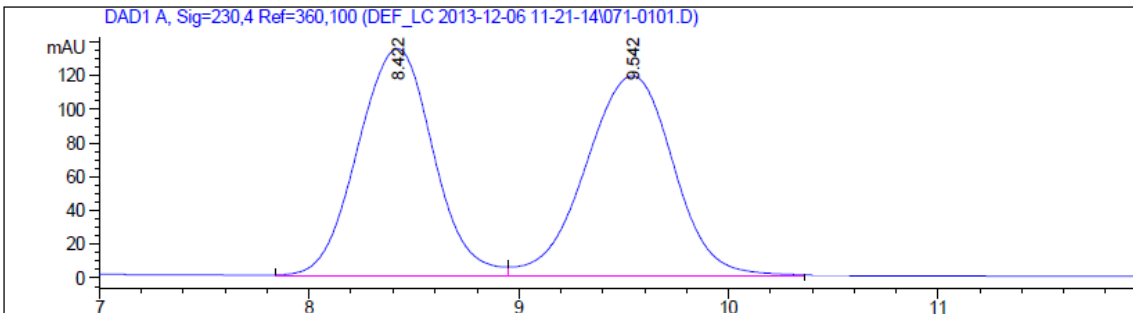
**Note: Carbons 2',3',4', 5' excluded for norspongiolactone due to structural differences.

Chiral HPLC Analysis of (±)-A3:



(±)-A3

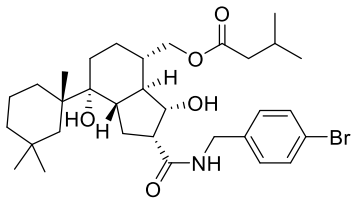
Chiralcel IA column: hexanes:*i*PrOH = 72:28, flow rate 1.0 mL/min, λ = 230 nm: t_1= 8.422 min, t_2= 9.542 min; racemic.



Signal 1: DAD1 A, Sig=230,4 Ref=360,100

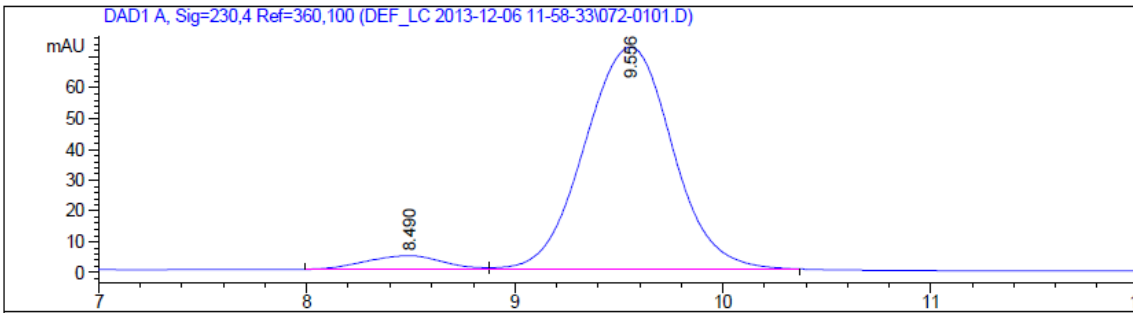
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.422	BV	0.3970	3358.42969	134.62383	49.4234
2	9.542	VB	0.4606	3436.79883	118.40112	50.5766

Chiral HPLC analysis of (+)-A3:



(+)-A3

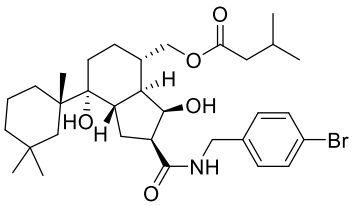
Chiralcel IA column: hexanes:*i*PrOH = 72:28, flow rate 1.0 mL/min, λ = 230 nm: t_{major}= 9.556 min, t_{minor}= 8.490 min; 90% *ee*.



Signal 1: DAD1 A, Sig=230,4 Ref=360,100

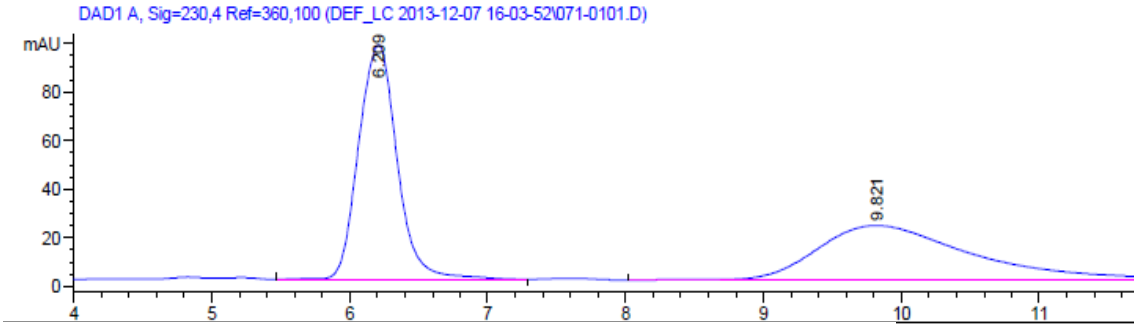
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.490	BV	0.4075	114.93547	4.44712	5.1236
2	9.556	VB	0.4658	2128.32886	72.23235	94.8764

Chiral HPLC analysis of A3:



(±)-A3

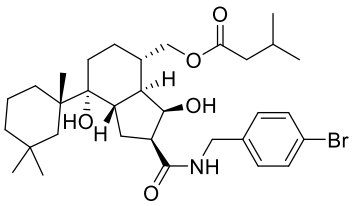
Chiralcel IA column: hexanes:*i*PrOH = 80:20, flow rate 1.0 mL/min, λ = 230 nm: t_1= 6.21 min, t_2= 9.82 min; racemic.



Signal 1: DAD1 A, Sig=230,4 Ref=360,100

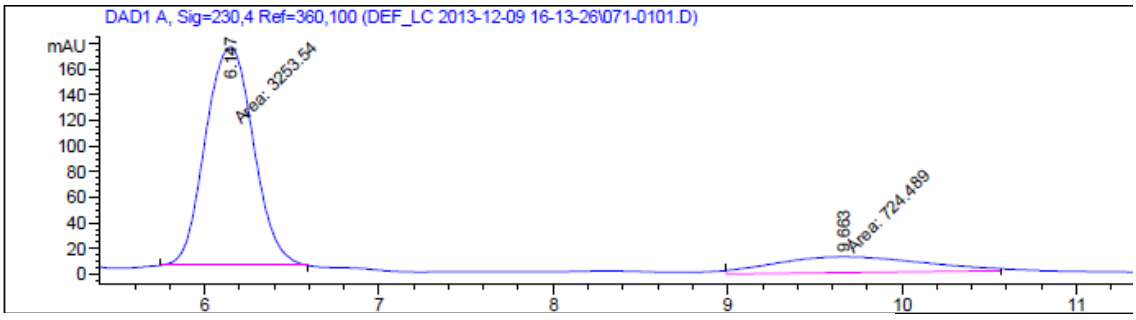
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.209	VV	0.3110	1909.91272	96.46696	52.5632
2	9.821	VB	1.1466	1723.63916	22.37854	47.4368

Chiral HPLC Analysis of (-)-A3:



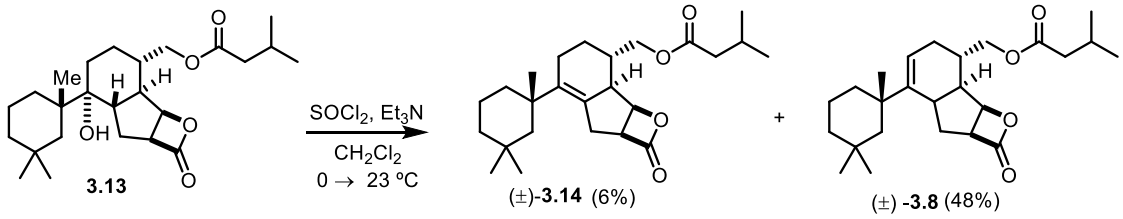
(-)-A3

Chiralcel IA column: hexanes:*i*PrOH = 80: 20, flow rate 1.0 mL/min, λ = 230 nm: t_{major}= 6.15 min, t_{minor}= 9.66 min; 64% *ee*.




```
Signal 1: DAD1 A, Sig=230,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.147 MM    0.3180 3253.54224  170.53874  81.7878
   2   9.663 MM    0.9853  724.48871   12.25501  18.2122
```

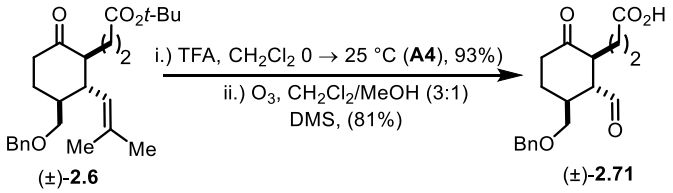


Regio, bis-epi spongiolactone (±)-3.8. β-lactone **(±)-3.13** (3.9 mg, 9.3 µmol, 1.0 equiv) was dissolved in CH_2Cl_2, Et_3N (6.8 µL, 49.0 µmol, 5.25 equiv) was added and the mixture cooled to 0 °C. Neat $SOCl_2$ (3.0 µL, 39.4 µmol, 4.25 equiv) was added dropwise over 2 min with a

microliter syringe. The reaction was warmed to ambient temperature (23 °C) and stirred until TLC indicated complete consumption of starting material (~12 h). The reaction was concentrated *in vacuo* and loaded directly onto a silica column for purification (SiO_2 0 → 30% EtOAc/hexanes) providing 0.2 mg of (±)-bis-epi spongiolactone (6%) and 1.8 mg of regio, bis-epi spongiolactone ((±)-**3.8**, 48%).

Regio, bis-epi spongiolactone (±)-3.8. R_f = 0.61 (3:7-EtOAc/hexanes); IR (thin film) 3064, 2954, 2925, 1821, 1807, 1637, 1267 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.49 (overlapping ddd, *J* = 5.0, 5.0, 2.5 Hz, 1H), 4.84 (app t, *J* = 3.5 Hz, 1H), 4.19 (dd, *J* = 11.0, 5.0 Hz, 1H), 3.96 (dd, *J* = 11.5, 6.0 Hz, 1H), 3.90 (d, *J* = 7.5, 4.0 Hz, 1H), 2.59-2.54 (m, 1H), 2.49 (dd, *J* = 12.5, 5.5 Hz, 1H), 2.29-2.23 (m, 1H), 2.16 (dd, *J* = 8.0, 1.5 Hz, 2H), 2.12-2.01 (m, 2H), 1.96-1.89 (m, 1H), 1.80-1.75 (m, 1H), 1.63 (overlapping ddd, *J* = 12.5, 12.5, 7.5 Hz, 1H), 1.58-1.45 (m, 3H), 1.42 (overlapping ddd, *J* = 14.0, 14.0, 3.0 Hz, 1H), 1.2-1.1 (m, 4H), 0.99 (s, 3H), 0.90 (d, 6H), 0.84 (s, 3H), 0.77 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.2, 171.2, 142.3, 126.8, 75.7, 67.4, 53.6, 50.0, 47.6, 43.4, 39.6, 39.4, 38.1, 33.2, 33.1, 31.4, 31.3, 29.7, 26.8, 26.7, 25.9, 25.6, 22.4 (2), 20.9; HRMS (ESI+) *m/z* calcd for $C_{25}H_{38}O_4Li$ [M+Li]: 403.2848, found: 403.2851.

Synthesis of simplified β-lactone cores (±)-18, (±)-19, and (±)-20 derived from *t*-butyl ester (±)-7.

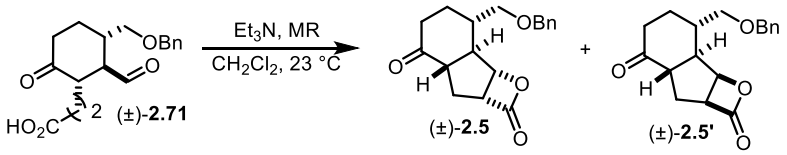


Aldehyde acid (±)-**2.71**. i) (*t*-butyl ester deprotection) A solution of ester (±)-**2.6** (3.0 g, 7.5 mmol, 1.0 equiv) in CH_2Cl_2 (100 mL) was cooled to 0 °C after which TFA (16.0 mL, 150 mmol, 20.0 equiv) was added, dropwise *via* syringe. The reaction was allowed to warm to room

temperature and proceed until TLC indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo* to afford a dark brown oil. Purification of the crude product by flash chromatography (SiO_2 0 → 30% EtOAc/hexanes) provided 2.4 g (93%) of the corresponding carboxylic acid as a brown oil: R_f = 0.50 (1:1-EtOAc:hexanes); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5H), 4.88 (dt, *J* = 10.0, 1.0 Hz, 1H), 4.43 (dd, *J* = 15.5, 12.0 Hz, 2H), 3.48 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.23 (dd, *J* = 9.0, 6.5 Hz, 1H), 2.49-2.39 (m, 3H), 2.34-2.24 (m, 3H), 2.23-2.18 (ddd, *J* = 13.5, 9.0, 2.5 Hz, 1H), 1.87-1.76 (m, 2H), 1.73 (d, *J* = 1.0 Hz, 3H), 1.71-1.61 (m, 2H), 1.54 (d, *J* = 1.0 Hz, 3H), O-H proton not observed; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 212.1, 179.1, 138.5, 134.6, 128.5(2), 127.7,127.6(2), 126.5, 73.3, 72.6, 53.6, 45.9, 42.7, 41.6, 32.1, 30.5, 25.9, 21.8, 18.7; IR (thin film) 3339, 3031, 2978, 1732, 1711, 1448, 1362 cm^{-1}; HRMS (ESI+) $C_{21}H_{28}O_4Li$ $[M+Li]^+$: 351.2148. Found: 351.2143.

ii) (ozonolysis) A solution of the intermediate carboxylic acid (1.40 g, 4.06 mmol, 1.0 equiv) was dissolved in a mixed solvent system of MeOH/CH_2Cl_2 (1:3, 6 mL/ 18 mL) and cooled to -78 °C. Ozone was bubbled through the reaction solution until a blue color persisted. Excess ozone was removed by blowing N_2 gas into the solution with stirring for 10 min. Dimethylsulfide (3.0 mL, 40.6 mmol, 10.0 equiv) was added by syringe at -78 °C and the reaction was slowly warmed to ambient temperature (23 °C) overnight to insure complete reduction of the ozonide. Purification by flash chromatography (SiO_2 0 → 60% EtOAc/hexanes) gave 790 mg (61%) of aldehyde acid **(±)-2.71** as a colorless oil: R_f = 0.32 (1:1-EtOAc:hexanes); IR (thin film) 3410, 3037, 2937, 2860, 2724, 1776, 1711, 1456, 1095 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.63 (d, *J* = 4.0 Hz, 1H), 7.36-7.27 (m, 5H), 4.43 (dd, *J* = 22.0, 12.0, Hz, 2H), 3.45 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.31 (dd, *J* = 9.5, 7.0 Hz, 1H), 2.73 (ddd, *J* = 11.5, 9.0, 2.5 Hz, 1H), 2.54-2.33 (m, 7H), 2.14 (ddd, *J* = 13.0, 8.5, 4.0 Hz, 1H), 1.91-1.84 (m, 1H), 1.65-1.55, 196 (m, 1H), O-H proton not observed; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 209.7, 200.7, 179.0, 137.7, 128.6(2), 127.9, 127.8(2),

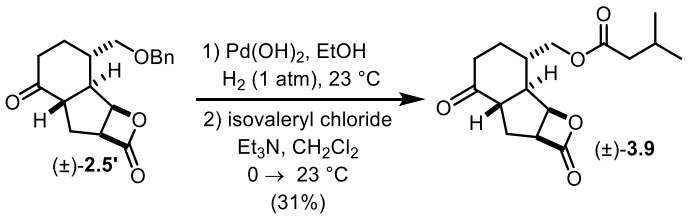
73.4, 72.5, 59.1, 46.7, 40.6, 38.7, 31.5, 29.2, 22.1; HRMS (ESI–) Calcd for $C_{18}H_{21}O_5$ $[M\text{-}H]^-$: 317.1389. Found: 317.1375.



β-lactones (±)-**2.5** and (±)-**2.5'.** To an oven-dried round-bottomed flask, modified Mukaiyama's reagent (1.48 g, 4.2 mmol, 3.0 equiv) was added and azeotroped with xylenes *in vacuo*. It was then dissolved in CH_2Cl_2 (30.0 mL) and Et_3N (1.0 mL, 7.1 mmol, 5.0 equiv) was added. A separate solution of aldehyde acid (±)-**2.71** (450 mg, 1.4 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (10.0 mL) and was subsequently added *via* syringe pump over 1 h. Purification (SiO_2 0 → 30% EtOAc/hexanes) provided a 1:3 mixture of diastereomeric β-lactones (±)-**2.5** (9%, 2.8 mg) and (±)-**2.5'** (30%, 9.1 mg).

Benzyl ether tricyclic β-lactone (±)-**2.5**. hexanes:EtOAc (1:1) R_f = 0.52 (2:3-EtOAc/hexanes); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.39-7.31 (m, 5H), 5.17 (app d *J* = 3.5 Hz, 1H), 4.56, 4.47 (ABq, *J* = 12.0 Hz, 2H), 3.96 (dd, *J* = 8.0, 3.5 Hz, 1H), 3.53 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.53 (dd, *J* = 9.0, 4.5 Hz, 1H), 3.01 (overlapping ddd, *J* = 13.0, 6.5, 6.5 Hz, 1H), 2.60 (dd, *J* = 12.0, 9.0 Hz, 1H), 2.48-2.41 (m, 1H), 2.38-2.32 (m, 2H), 2.12-2.08 (m, 1H), 1.96 (overlapping ddd, *J* = 13.0, 13.0, 8.0 Hz, 1H), 1.63-1.58 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 209.9, 169.8, 137.8, 128.7(2), 128.1, 127.8(2), 79.0, 73.7, 73.2, 54.9, 49.1, 48.2, 37.1, 33.3, 29.9, 28.9; IR (thin film) 2939, 2865, 1826, 1711, 1619 cm^{-1}; HRMS (ESI+) Calcd for $C_{18}H_{21}O_4$ $[M+H]^+$: 301.1440. Found: 301.1426.

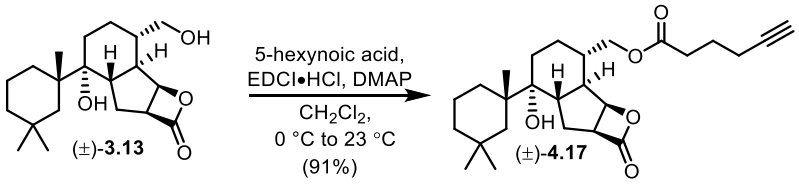
Diastereomeric benzyl ether tricyclic β-lactone (±)-**2.5'**: hexanes:EtOAc (1:1) R_f = 0.66 (1:1-EtOAc/hexanes); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.37-7.28 (m, 5H), 4.95 (app t, *J* = 4.0 Hz, 1H), 4.52, 4.48 (ABq, *J* = 12.0 Hz, 2H), 3.87 (dd, *J* = 8.0, 3.5 Hz, 1H), 3.59 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.50 (dd, *J* = 9.5, 5.5 Hz, 1H), 2.82-2.76 (overlapping ddd, *J* = 12.5, 12.5, 5.5 Hz, 1H), 2.42-2.39 (m, 2H), 2.33-2.26 (m, 1H), 2.14 (overlapping ddd, *J* = 16.5, 16.5, 5.5, 2H), 1.82 (ddd, *J* = 14.0, 12.0, 8.0, Hz, 1H), 1.75-1.64 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 209.2, 170.8, 138.1, 128.6(2), 127.9, 127.7(2), 76.1, 73.4, 71.9, 54.5, 51.9, 50.2, 40.6, 37.0, 30.5, 24.8; IR (thin film): 3030, 2928, 2863, 1824, 1717, 1453, 1119 cm^{-1}; HRMS (ESI+) Calcd for $C_{18}H_{21}O_4$ $[M+H]^+$: 301.1440. Found: 301.1435.



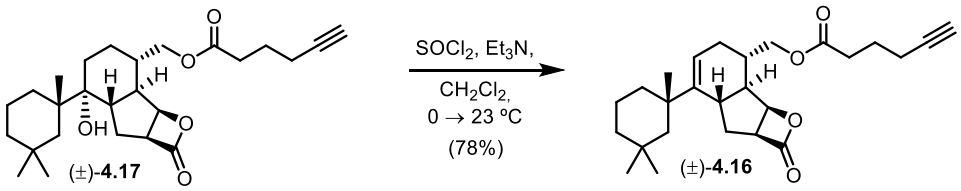
Isovaleric ester (±)-**3.9.** To a solution of (±)-**2.5'** (10.0 mg, 0.033 mmol, 1.0 equiv) in EtOH (2 mL), was added $Pd(OH)_2$ (2.8 mg, 20 wt%, 0.0019 mmol, 0.05 equiv). The contents of the flask were then evacuated and flushed with H_2 from a balloon three times consecutively and the reaction was then allowed to proceed with vigorous stirring until TLC indicated complete consumption of starting materials. The reaction mixture was then passed over a pad of Celite, washed with EtOH, and the crude filtrate was concentrated *in vacuo*. The material was carried on directly without further purification.

To primary alcohol **A5** in CH_2Cl_2 (2.68 mL) at 0 °C, Et_3N (65 µL, 0.468 mmol, 3.5 equiv) was added followed by the dropwise addition of isovaleryl chloride (41 µL, 0.334 mmol, 2.5 equiv). The reaction was allowed to proceed until TLC indicated the reaction's completion. Purification utilized preparatory TLC in 30% EtOAc/hexanes developed three times and afforded 3.1 mg

(32% over two steps) of the desired isovaleric ester (±)-**3.9**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.01 (app t, *J* = 3.5, 3.5 Hz, 1H), 4.30 (dd, *J* = 11.5, 5.0 Hz, 1H), 3.99 (dd, *J* = 11.5, 6.0 Hz, 1H), 3.92 (dd, *J* = 8.0, 3.5 Hz, 1H), 2.77 (overlapping ddd, *J*= 11.0, 11.0, 5.0 Hz, 1H), 2.43 (dd, *J* = 9.0, 4.5 Hz, 2H), 2.40-2.32 (m, 1H), 2.21-2.14 (m, 4H), 2.10-2.02 (m, 1H), 1.89-1.82 (m, 1H), 1.62-1.53 (m, 2H), 0.94 (overlapping doublets, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 208.4, 172.9, 170.2, 75.6, 65.4, 54.5, 52.1, 50.1, 43.2, 40.3, 36.2, 30.1, 25.6, 24.6, 22.4 (2); IR (thin film): 2961, 2925, 1827, 1720 cm^{-1}; HRMS (ESI+) Calcd for $C_{18}H_{20}O_4Li$ [M+Li]: 301.1627. Found: 301.2064.



Alkynyl alcohol (±)-**4.17.** Primary alcohol (±)-**3.13** (0.131 g, 0.299 mmol, 1 equiv) was dissolved in CH_2Cl_2 (0.3 mL). To this solution, DMAP (3.7 mg, 0.029 mmol, 10 mol%) was added followed by 5-hexynoic acid (33 μL, 0.299 mmol, 1 equiv). After cooling to 0 ˚C, DCC (68 mg, 0.33 mmol, 1.1 equiv) was added and the reaction was allowed to gradually warm to room temperature and proceed overnight. The next day, the white precipitate that had formed was removed by filtration through a Celite plug which was subsequently washed with CH_2Cl_2 (2 mL). The organics were washed with 0.5 N HCl (2 x 2 mL) followed by $NaHCO_3$ (2 mL). dried (Na_2SO_4), and concentrated *in vacuo*. The resulting product (90%, 0.144 g) was taken on directly to the next reaction.



Alkynyl probe (±)-**4.16.** Tertiary alcohol (±)-**4.17** (56.6 mg, 0.131 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 and Et_3N and cooled to 0 ˚C. $SOCl_2$ (40.4 μL, 0.557 mmol, 4.25 equiv) was added via microliter syringe and the reaction was allowed to proceed overnight. The next day it was concentrated directly and purified by flash column chromatography. Purification (SiO_2 0 → 30% EtOAc/hexanes) provided alkynyl probe (±)-**4.16** (78%, 4.2 mg).

Alkynyl probe (±)-4.16. hexanes:EtOAc (3:1) R_f = 0.7; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.56 (app s, 1H), 4.95 (app t, J = 4.0 Hz, 1H), 4.23 (dd, J = 11.5, 5.0 Hz, 1H), 3.98 (dd, J = 11.0, 6.0 Hz, 1H), 3.83, (dd, J = 8.5, 4.0 Hz, 1H), 2.45 (app t, J = 8.0 Hz, 2H), 2.33-2.23 (m, 6H), 2.20-2.17 (m, 1H), 2.12-2.06 (m, 1H), 1.95 (app t, J = 2.5 Hz, 2H), 1.89-1.82 (m, 6H), 1.75-1.74 (m, 2H), 1.32-1.21 (m, 2H), 1.12 (s, 3H), 0.89 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.1, 171.2, 145.3, 119.3, 83.2, 74.6, 69.2, 66.7, 55.0, 49.7, 49.2, 39.9, 39.8, 39.4, 38.0, 33.5, 32.8, 32.2, 31.8, 31.4, 30.4, 30.1, 29.7, 23.5, 19.8, 17.8; IR (thin film): 3538, 3310, 1821, 1724 cm^{-1}; HRMS (ESI+) Calcd for $C_{26}H_{37}O_4$ $[M+H]^+$: 412.2614. Found: 413.2511.

Figure A1 Single Crystal X-ray Crystallographic Data for Ester (±)-**2.77.**
CCDC 970423 ((±)-**13**) contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

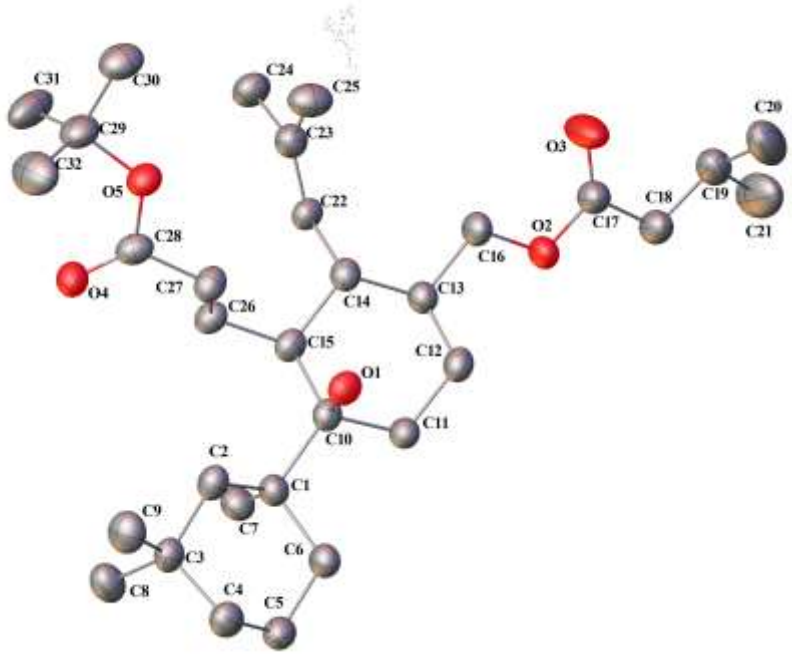


Table A5. Crystal data and structure refinement for DRB_NH_130228_G_IE.

Identification code	drb	
Empirical formula	C32 H56 O5	
Formula weight	520.77	
Temperature	110(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P21/c	
Unit cell dimensions	a = 17.128(3) Å	α= 90°.
	b = 10.413(2) Å	β= 119.543(12)°.
	c = 20.540(4) Å	γ = 90°.
Volume	3187.1(10) $Å^3$	
Z	4	
Density (calculated)	1.085 Mg/m^3	

Table A5 Continued.

Absorption coefficient	0.556 mm^{-1}
F(000)	1152
Crystal size	0.05 x 0.04 x 0.01 mm^3
Theta range for data collection	2.97 to 60.00°.
Index ranges	-19<=h<=19, -11<=k<=11, -23<=l<=22
Reflections collected	19254
Independent reflections	4735 [R(int) = 0.1156]
Completeness to theta = 60.00°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9945 and 0.9727
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4735 / 0 / 346
Goodness-of-fit on F^2	1.024
Final R indices [I>2sigma(I)]	R1 = 0.0624, wR2 = 0.1450
R indices (all data)	R1 = 0.1249, wR2 = 0.1721
Extinction coefficient	0.0017(2)
Largest diff. peak and hole	0.274 and -0.232 e.$Å^{-3}$

Table A6. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for DRB_NH_130228_G_IE. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	4527(2)	1502(2)	1639(1)	34(1)
O(2)	1637(2)	1084(2)	-1244(1)	40(1)
O(3)	218(2)	1662(4)	-1637(2)	79(1)
O(4)	4330(2)	-1366(2)	3568(1)	40(1)
O(5)	3194(2)	52(2)	3248(1)	39(1)
C(1)	5576(2)	-295(3)	1764(2)	32(1)
C(2)	5945(2)	-119(4)	2615(2)	36(1)
C(3)	6960(2)	-303(3)	3163(2)	35(1)
C(4)	7498(3)	473(4)	2887(2)	40(1)

Table A6 Continued.

	x	y	z	U(eq)
C(5)	7208(2)	168(4)	2078(2)	40(1)
C(6)	6212(2)	446(4)	1564(2)	36(1)
C(7)	5553(2)	-1709(3)	1540(2)	39(1)
C(8)	7261(3)	-1712(4)	3278(2)	47(1)
C(9)	7138(3)	200(4)	3924(2)	49(1)
C(10)	4585(2)	306(3)	1300(2)	31(1)
C(11)	4364(2)	624(3)	496(2)	33(1)
C(12)	3438(2)	1206(3)	23(2)	36(1)
C(13)	2700(2)	328(3)	-31(2)	33(1)
C(14)	2858(2)	-39(3)	745(2)	33(1)
C(15)	3826(2)	-578(3)	1255(2)	32(1)
C(16)	1782(2)	936(4)	-494(2)	39(1)
C(17)	809(3)	1424(4)	-1774(2)	45(1)
C(18)	721(2)	1430(4)	-2532(2)	46(1)
C(19)	-210(3)	1766(4)	-3164(2)	55(1)
C(20)	-406(3)	3192(5)	-3154(3)	82(2)
C(21)	-314(3)	1380(6)	-3905(3)	88(2)
C(22)	2176(2)	-1028(3)	675(2)	32(1)
C(23)	1520(2)	-948(3)	841(2)	39(1)
C(24)	911(3)	-2068(4)	736(2)	46(1)
C(25)	1297(3)	239(4)	1142(2)	51(1)
C(26)	3917(2)	-1053(3)	2002(2)	32(1)
C(27)	3866(3)	9(3)	2500(2)	37(1)
C(28)	3836(2)	-525(4)	3161(2)	36(1)
C(29)	3010(3)	-267(4)	3860(2)	48(1)
C(30)	2246(3)	647(4)	3708(3)	54(1)
C(31)	2698(3)	-1647(4)	3779(3)	67(1)
C(32)	3831(3)	12(5)	4606(2)	68(1)

Cell culture

K562 (chronic myelogenous leukemia (CML)) cell line was cultured in RPMI-1640 medium (Sigma R7509) supplemented with 2 mM L-glutamine and 10% heat-inactivated FCS. Medium used for MTT assay did not contain Phenol Red. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2.

Cytotoxicity assay (MTT assay)[3]

Cells at exponential phase were used for the assay. Cells were diluted with a fresh culture medium to the concentration $1.6 \cdot 10^5$ cells/mL. 50 µL cell suspension were plated in a 96-well round-bottom transparent plate, so that cell density was $8 \cdot 10^3$ cells per well. Also, wells not containing cells were included in the assay to subtract blank absorbance resulting from medium, MTT and a lysis buffer. Directly before the treatment, compounds **2.1**, **2.5**, **2.5'**, **2.79**, **3.8** and **3.9** were diluted 1:49 (v/v) from DMSO stocks (stored at -80° C) with a pre-warmed culture medium. 50 µL testing compound solution were added to the 50 µL cells in triplicate wells. Final volume in each well was 100 µL, cell density was 8×10^4 cells/mL and final compound concentration ranged between 0.01 and 1000 µM. After 24 h exposure, 20 µL MTT solution (5 mg/mL in PBS, sterile filtered) [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma M5655)] were added to each well and mixed well into the medium. The plates were incubated at 37 °C, 5% CO_2 for 4 h in the dark to allow MTT to be metabolized. Then, 50 µL 'triplex lysis solution' (10% SDS (w/v), 5% isobutanol (v/v), 0.012 M HCl in distilled H_2O) were added to each well and mixed well into the medium. The plates were incubated overnight (14-16 h) at 37 °C, 5% CO_2. The complete dissolution of the formazan salt (metabolic product of

[3] C. Qing, Z. H. Miao, L. J. Tong, J. S. Zhang, J.Ding *Acta Pharmacol. Sin.* **2003**, *24*, 415-421.

MTT) was checked under the microscope and the optical density was measured at 570 nm by a TECAN Infinite® M200 Pro. All biological experiments were performed with three independent replicates. Average blank absorbance (medium, MTT solution and lysis buffer) was subtracted from average absorbance of wells containing cells. Percent inhibition of metabolic activity (cells viability) was calculated as a fraction of control (cells treated only with the DMSO (vehicle)). IC_{50} values (when applicable) were calculated using sigmoidal dose response curve fitting algorithm in OriginPro 8.5.1 (OriginLab Corporation).

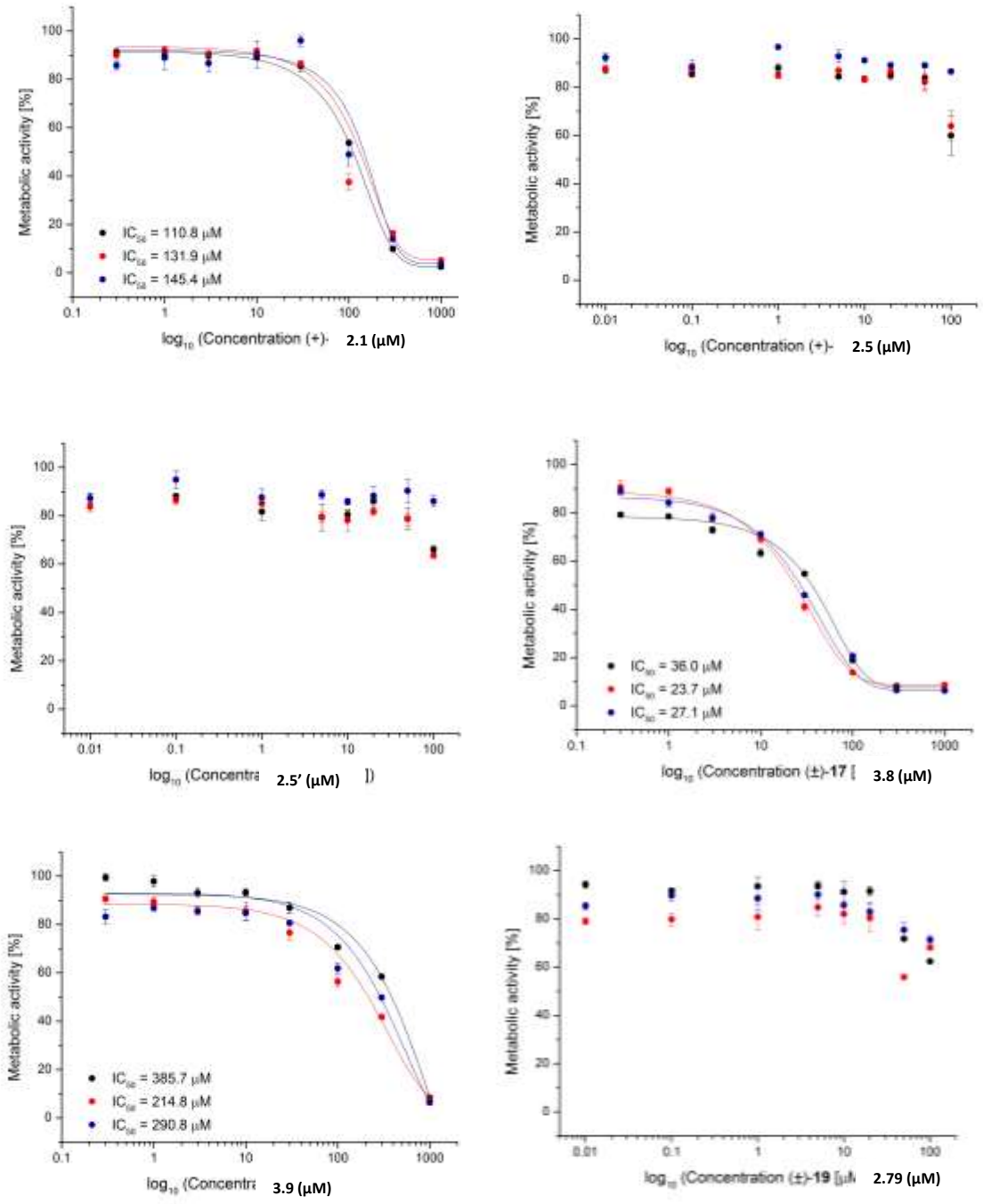
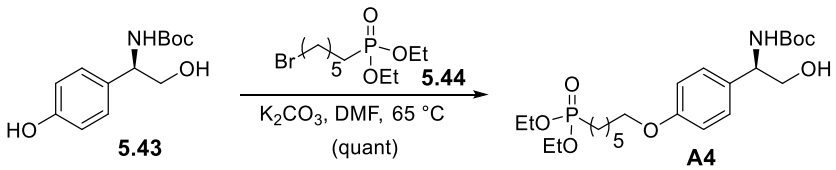
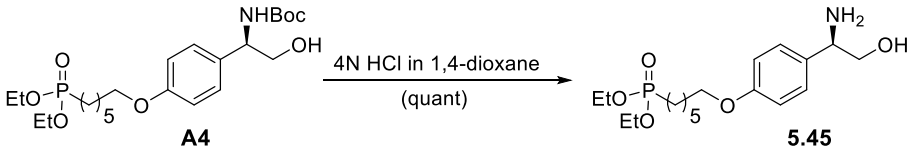


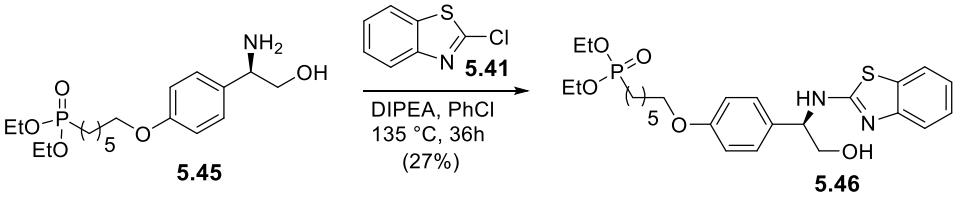
Figure A2. Dose response fit of cell metabolic activity (MTT assay) upon treatment with different concentrations of spongiolactone and derivatives, (+)-**2.1**, (+)-**2.5**, (±)-**2.5'**, (±)-**3.8**, (±)-**2.79** and (±)-**3.9**, to determine IC_{50} values.



Phosphonate ester A4. To a pre-weighed mixture of phenol **5.43** (1.50 g, 5.92 mmol, 1.0 equiv) and phosphonate ester bromide **5.44** (2.30, 7.7 mmol, 1.3 equiv) in DMF (16 mL), K_2CO_3 (1.3 g, 9.48 mmol, 1.6 equiv) was added. The reaction was heated in an oil bath to 65 °C and kept at this temperature for 18 h. The reaction was quenched with saturated NH_4Cl (16 mL) and water (10 mL) was added to completely dissolve all solids. The reaction mixture was then extracted with EtOAc (3 x 50 mL). The combined organics were washed with water (100 mL) and brine (100 mL), dried with Na_2SO_4, and concentrated *in vacuo* to provide 2.80 g (quant) of phosphonate ester **A4** as a brown oil which was taken on directly without further purification: TLC (EtOAc:hexanes, 1:1 *v*/*v*): R_f = 0.2 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.16 (d, *J* = 9.0 Hz, 2H), 6.81 (d, *J* = 9.0 Hz, 2H), 5.43 (app d, *J* = 18.0 Hz, 1H), 4.66 (br s, 1H), 4.06-4.00 (m, 4H), 3.89 (t, *J* = 6.5 Hz, 2H), 3.75 (br m, 2H), 1.73-1.66 (m, 4H), 1.61-1.56 (m, 2H), 1.43-1.39 (m, 14H), 1.28 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): 158.6, 156.2, 129.5, 127.8 (2), 114.8 (2), 79.9, 67.9, 67.0, 61.6 (2), 56.5, 30.3 ($J_{C\text{-}P}$=17.0 Hz), 29.1, 28.5 (3), 25.7 (t, $J_{C\text{-}P}$ = 62.5 Hz), 22.5, 22.4, 16.6 (2); ^{31}P NMR (202.4 MHz, $CDCl_3$): 32.5; IR (thin film): 3428, 2982, 1712, 1514, 1250 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{23}H_{39}NNaO_7P$ $[M+Na]^+$: 496.2440, found: 496.2431.

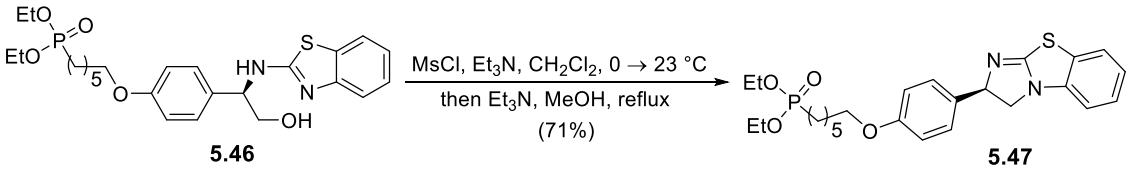


Alcohol 5.45. Phosphonate ester **A4** (2.80 g, 5.92 mmol, 1.0 equiv) was dissolved in 4N HCl in 1,4-dioxane (29.5 mmol, 7.38 mL, 5.0 equiv) and stirred at 23 °C for 3 hours at which point TLC showed complete consumption of starting material. The reaction was diluted with EtOAc (5 mL) and the aqueous layer was basified with 1N NaOH solution to achieve a pH of 10. The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (30 mL), dried with Na_2SO_4, and concentrated *in vacuo* to cleanly provide amino alcohol **5.45** (2.2 g, quant) as a brown oil which was taken on without further purification: TLC (EtOAc): R_f = 0.1 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J*= 8.5 Hz, 2H), 4.06-4.00 (m, 4H), 3.88 (t, *J* = 6.5 Hz, 2H), 3.68-3.66 (m, 1H), 3.58 (app t, *J* = 7.0 Hz, 1H), 3.53 (app t, *J* = 9.5 Hz, 1H), 2.92 (br s, 2H), 1.74-1.65 (m, 4H), 1.61-1.50 (m, 2H), 1.41-1.38 (m, 5H), 1.27 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): 158.6, 133.8, 127.8 (2H), 114.7 (2), 67.9, 67.7, 61.6, 61.5, 56.9, 30.3 ($J_{C\text{-}P}$=17.0 Hz), 29.1, 25.7 (t, $J_{C\text{-}P}$ = 71.5 Hz), 22.5, 22.4, 16.6 (2); ^{31}P NMR (202.4 MHz, $CDCl_3$): 32.4; IR (thin film): 3419, 2933, 1612, 1517, 1247, 1056 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{18}H_{33}NO_5P$ $[M+H]^+$: 374.2096, found: 374.2112.



Alcohol 5.45.[1] Amino alcohol **5.45** (2.20 g, 5.92 mmol, 1.0 equiv) was weighed into a high pressure vessel to which chlorobenzene (2.85 mL), *i*Pr_2Net (1.34 mL, 7.7 mmol, 3.9 equiv), and

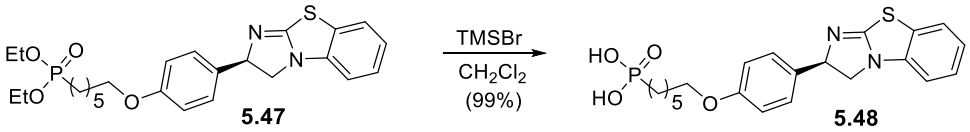
1-chlorobenzothiazole (0.27 mL, 2.09 mmol, 1.06 equiv). The reaction was heated in an oil bath to 135 °C and kept at this temperature for 18 h. Purification by flash column chromatography (SiO_2, 10 → 100% EtOAc/hexanes, 0 → 10% MeOH/CH_2Cl_2) gave 0.269 g (27%) of alcohol **5.46** as a brown oil: TLC (MeOH:CH_2Cl_2, 1:9 *v*/*v*): R_f = 0.6 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43 (dd, *J* = 8.0, 3.5 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 7.19 (app t, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.98 (app t, *J* = 7.5 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.70 (dd, *J* = 7.5, 4.0 Hz, 1H), 4.05-4.00 (m, 4H), 3.85 (t, *J* = 7.0 Hz, 2H), 3.80 (dd, *J* = 11.5, 7.5 Hz, 1H), 3.57 (app t, *J* = 6.5 Hz, 1H), 1.70-1.64 (m, 4H), 1.58-1.52 (m, 2H), 1.41-1.38 (m, 6H), 1.27 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): 167.9, 158.8, 156.3, 151.7, 130.7, 128.2 (2), 125.9, 121.6, 120.8, 118.8, 114.7 (2), 67.8, 66.9, 62.6, 61.6 (2), 30.3 (d, $J_{C\text{-}P}$=17.0 Hz), 29.0, 28.4, 25.6 (t, $J_{C\text{-}P}$ = 63.4 Hz), 22.4 (d, $J_{C\text{-}P}$ = 5.0 Hz), 16.5 (2); IR (thin film): 3389, 3252, 2936, 1712, 1244 cm^{-1}; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{36}N_2O_5P$ $[M+H]^+$: 507.2083, found: 507.2107.



5.46 **5.47**

Phosphonate ester, (+)-benzotetramisole 5.47. Alcohol **5.46** (0.305 g, 0.601 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (6.0 mL) and Et_3N (0.25 mL, 1.80 mmol, 3.0 equiv) and cooled to 0 °C in an ice bath. Methanesulfonyl chloride (70 µL, 0.90 mmol, 1.5 equiv) was added dropwise and the reaction was kept at 0 °C for 30 minutes. The ice bath was removed and the reaction warmed to room temperature and proceeded for 2 hours. After this time, MeOH (0.25 mL, 6.17 mmol, 10.3 equiv) was added followed by Et_3N (0.25 mL, 1.80 mmol, 3.0 equiv) and the reaction was heated to reflux and proceeded overnight. Purification by flash column chromatography (SiO_2, 10 → 100% EtOAc/hexanes, 0 → 10% MeOH/CH_2Cl_2) gave 0.208 g

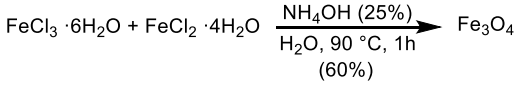
(71%) of phosphonate ester **5.47** as a brown oil: TLC (MeOH:CH_2Cl_2, 1:9 *v*/*v*): R_f = 0.6 ; $[\alpha]_D^{20}$ = +63.3 (*c* = 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): 7.23 (d, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.0 Hz, 2H), 7.11 (app t, *J* = 7.5 Hz, 1H), 6.90 (app t, *J* = 8.0 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.61 (d, *J* = 8.0 Hz, 1H), 5.53 (app t, *J* = 9.5 Hz, 1H), 4.18 (app t, *J* = 10.0 Hz, 1H), 4.07-3.99 (m, 4H), 3.86 (t, *J* = 6.0 Hz, 2H), 3.61 (app t, *J* = 8.5 Hz, 1H), 1.69-1.63 (m, 4H), 1.58-1.52 (m, 2H), 1.38-1.35 (m, 6H), 1.24 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): 166.5, 158.5, 136.9, 134.7, 127.5 (2), 126.6, 123.1, 121.5, 114.6 (2), 108.6, 74.4, 67.7, 61.3 (2), 52.5, 30.2 (d, $J_{C\text{-}P}$=16.9 Hz), 28.9, 28.8, 25.5 (t, $J_{C\text{-}P}$ = 70.0 Hz), 22.3 (2), 16.4 (2); IR (thin film): 2939, 1757, 1603, 1511, 1472, 1244 cm^{-1}; ^{31}P NMR (202.4 MHz, $CDCl_3$): 32.4; HRMS (+ESI) *m*/*z* calcd for $C_{25}H_{34}N_2O_4PS$ $[M+H]^+$: 489.1977, found: 489.1998.



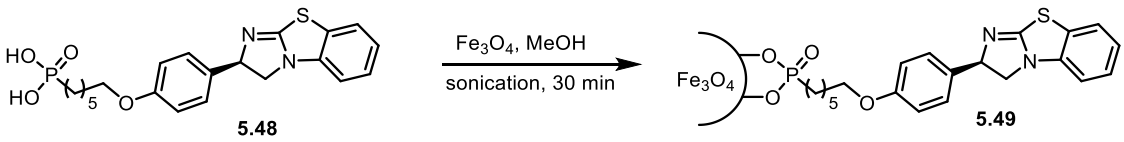
Phosphonic acid 5.48. To a solution of phosphonate ester (0.204 g, 0.420 mmol, 1.0 equiv) in CH_2Cl_2 (4.2 mL) at 23 °C was added trimethylsilyl bromide (0.276 mL, 2.10 mmol, 5.0 equiv). The reaction was allowed to proceed overnight after which time TLC indicated complete consumption of starting material. The reaction was concentrated, taken up in MeOH (3 mL), and stirred for 3 h. After this time, it was concentrated *in vacuo* and taken up in $CHCl_3$ (5 mL) and water (5 mL). A brown, viscous oil layer formed which was separated from both the $CHCl_3$ and water layers and dried *in vacuo* to provide pure phosphonic acid **5.48**. TLC (MeOH, 1:1 *v*/*v*): R_f = 0.1; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.59 (dd, J = 7.5, 1.0 Hz, 1H), 7.34 (td, J = 7.0, 1.0 Hz, 1 H), 7.20-7.18 (m, 2H), 7.15 (d, J = 9.0 Hz, 2H), 6.71 (d, J = 8.5 Hz, 2H), 5.78 (dd, J = 11.0, 8.5 Hz, 1H), 4.84-4.79 (m, 1H), 4.12 (dd, J = 10.5, 8.5 Hz, 1H), 3.74 (t, J = 6.5 Hz, 1H), 1.55-1.42

(m, 6H), 1.30-1.20 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$): 169.5, 160,1, 137.5, 134.2, 128.0 (2), 127.4, 125.7, 124.4, 115.4 (2), 112.6, 68.0, 66.7, 53.5, 49.7, 30.2, 28.9 (2), 25.6, 22.5; IR (thin film): 3422, 2933, 1612, 1514, 1466, 1247 cm^{-1}; ^{31}P NMR (202.4 MHz, $CDCl_3$): 17.7; HRMS (+ESI) *m/z* calcd for $C_{21}H_{26}ClN_2O_4PS$ $[M\text{-}Cl]^+$: 433.1351, found: 433.1357.

Magnetite preparation.



$FeCl_3 \cdot 6\ H_2O$ (2.335 g, 8.6 mmol, 2.0 equiv) and $FeCl_2 \cdot 4\ H_2O$ (0.860 g, 4.3 mmol, 1.0 equiv) were weighed into a 250 mL round-bottom flask and dissolved in distilled water (80 mL). The solution was heated to 90 ˚C and evacuated/flushed with N_2 for 5 cycles. NH_4OH (25%, 3.0 mL) was added, dropwise, with vigorous stirring during which time a black precipitate formed. Magnetite formation was allowed to proceed for 1 hour after which time magnetic decantation with a neodymium magnet was used to isolate the product. The MNP were washed with distilled water (4 x 50 mL) until the pH of the supernatant was neutral. Finally, the magnetite was washed with EtOH (2 x 50 mL) and dried *in vacuo* overnight to provide magnetite (1.98 g, 60% yield) as a fine, black powder.

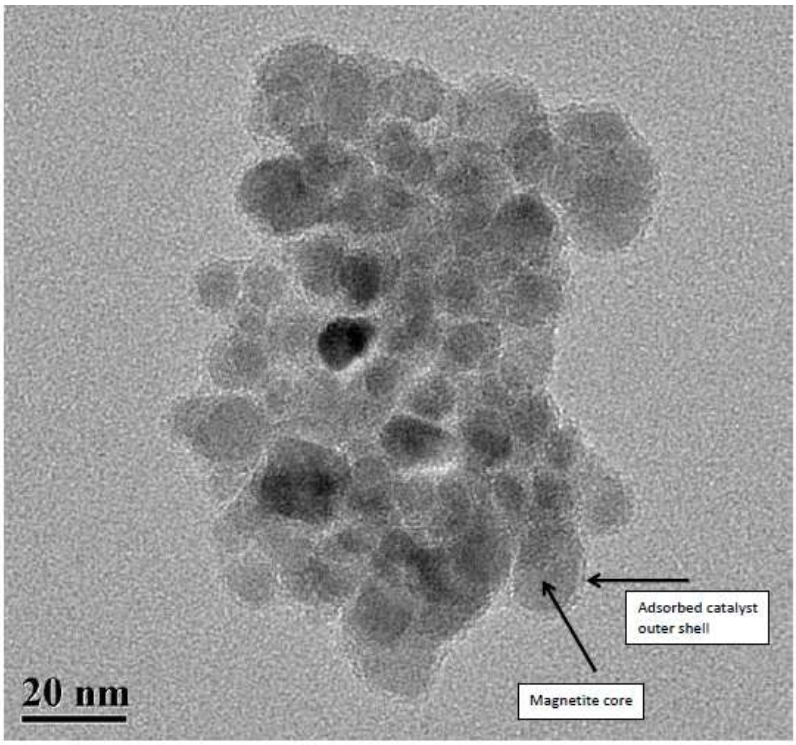


Loading of catalyst by sonication.

A solution of phosphonic acid-BTM derivative **5.48** (0.1255 g, 0.291 mmol, 1 equiv) was prepared in methanol (2.9 mL). Freshly prepared Fe_3O_4 (45.0 mg, 0.19 mmol, 0.65 equiv) was added and the homogeneous solution was sonicated for 30 minutes at 23 ˚C. After this time, a

neodymium magnet was used to attract the magnetite to the bottom of the vial. When the solution became clear, the supernatant was removed by pipette. The vial was removed from the magnet and the MNP were washed with MeOH (3 mL) to remove unreacted catalyst. The washing/magnetic decantation process was repeated for a total of three times and the final MNP were dried *in vacuo* overnight. IR (thin film): 2860, 1606, 1511, 1469, 1244, 983 cm^{-1}.

Transmission Electron Microscopy (TEM) of 5.59.

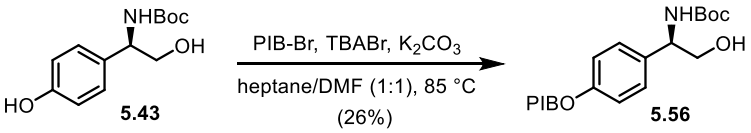


Determination of catalyst loading.

A 52.06 mM stock solution of $NaHPO_4$ was prepared by adding 73.9 mg (0.52 mmol) of $NaHPO_4$ to a volumetric flask and diluting to a final volume of 10 mL. Separately, phosphonic acid-BTM derivative **5.48** (8.1 mg, 0.19 mmol) was dissolved in concentrated HCl (12 M, 0.2 mL). To this solution, NaOH (12 M, 0.2 mL) was added to adjust the pH of the solution to 13

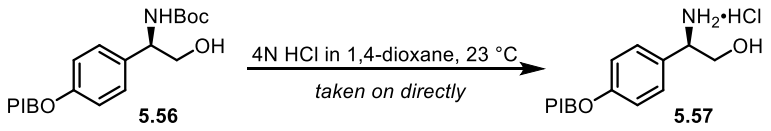
causing a salt to precipitate which was removed by filtration through Celite in a disposable pipette. The Celite and filter cake were rinsed with MeOH (0.2 mL) and enough water to give a final volume of 1 mL.

An NMR sample was then prepared with the following components: 50 μL of $NaHPO_4$ stock solution (52.06 mM), 50 μL of NaOH (12 M), 500 μL of MNP solution, and 22 μL of D_2O. Catalyst loading was determined to be 0.067 mmol catalyst/1 g of sample by utilizing the relative ^{31}P peak intensities from the MNP and $NaHPO_4$.

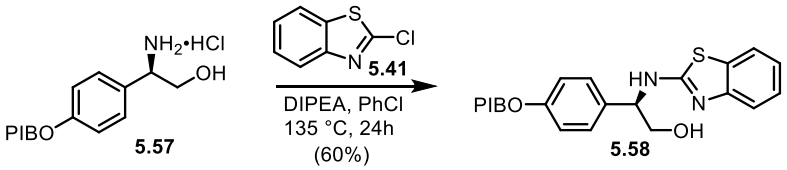


Polyisobutylene ether 5.56. Polyisobutylene bromide (3.32 g, 3.16 mmol, 2.0 equiv) was weighed and dissolved in heptane (8 mL). Phenol **5.43** (0.40 g, 1.58 mmol, 1.0 equiv) was added to the polymer solution along with DMF (8 mL) followed by K_2CO_3 (1.44 g, 3.16 mmol, 2.0 equiv) and tetrabutylammonium bromide (0.50 g, 1.58 mmol, 1.0 equiv). The reaction was heated in an oil bath to 80 °C and kept at this temperature for 18 h. Upon cooling, the heptane layer was separated. Water (50 mL) was added to dissolve all solids in the aqueous layer which was further extracted with hexanes (3 x 50 mL). The combined organics were washed with water (100 mL) and brine (100 mL), dried with Na_2SO_4, and concentrated *in vacuo* to provide 0.49 g (26%) of PIB-ether **5.56**. Purification by MPLC (0 → 100% EtOAc/hexanes): TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.5 ; ^1H NMR (500 MHz, $CDCl_3$): δ 7.16 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 5.16 (br s, 1H), 4.68 (br s, 1H), 3.76-3.72 (m, 3H), 3.57 (app t, *J* = 8.0 Hz, 1H), 1.44-1.36 (m), 1.13-1.02 (m); ^{13}C NMR (125 MHz, $CDCl_3$): 159.0, 156.3, 131.3, 128.1,

127.4, 115.1, 114.6, 80.0, 67.2, multiple peaks between 59.6 and 58.3, 56.9, 49.8, multiple peaks between 38.3 and 28.4, 20.5; IR (thin film): 3431, 2964, 1682, 1511, 1387, 1244 cm^{-1}.

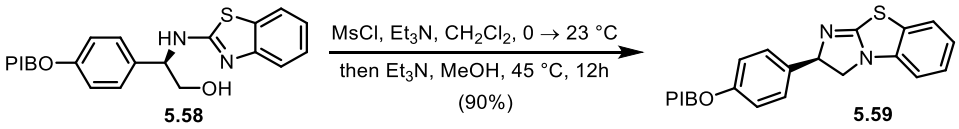


PIB Amino Alcohol 5.56. PIB ether **5.56** (0.49 g, 0.41 mmol, 1.0 equiv) was weighed and dissolved in 1,4-dioxane (3 mL). To this, 4N HCl in 1,4-dioxane (6.6 mL) was added at 23 ˚C. The reaction was allowed to proceed overnight. After this time, the starting material was completely consumed as determined by TLC, the reaction was concentrated *in vacuo* to provide 0.45 g (quant) of amino alcohol **5.57** which was taken on directly as the hydrochloride salt to the next reaction. TLC (EtOAc:hexanes, 3:7 *v*/*v*): R_f = 0.05; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (d, *J* = 7.5 Hz, 2H), 6.84 (d, *J* = 8.0 Hz, 2H), 3.96 (br s, 1H), 3.73 (app t, *J* = 8.0 Hz, 1H), 3.67 (br m, 1H), 3.58 (app t, *J* = 8.0 Hz, 1H), 3.50 (br s, 1H), 1.39-1.30 (m), 1.1-0.96 (m); ^{13}C NMR (125 MHz, $CDCl_3$): 158.8, 134.5, 127.9, 127.3, 114.9, 114.4, 74.7, 74.3, multiple peaks between 59.6 and 56.8, 49.8, multiple peaks between 38.3 and 29.3, 20.5**;** IR (thin film): 3383, 2951, 1745, 1615, 1511, 1469, 1366, 1238 cm^{-1}.



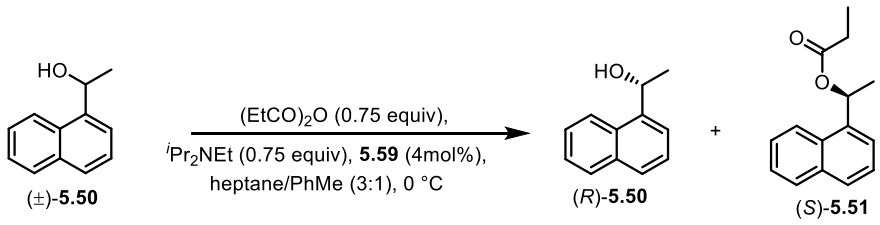
Aminobenzothiazole 5.58. Amino alcohol hydrochloride salt **5.57** (0.45 g, 0.41 mmol, 1.0 equiv) was weighed, dissolved in CH_2Cl_2 (2 mL) and transferred to a sealed pressure tube. The solvent was evaporated with a stream of N_2. Chlorobenzene (0.5 mL) was added followed by Hünig's base (0.28 mL, 1.6 mmol, 3.9 equiv) and the dropwise addition of 2-

chlorobenzothiazole (70 μL, 0.43 mmol, 1.06 equiv). N_2 was blown over the reaction mixture for 5 minutes after which time the tube was sealed and heated in an oil bath to 135 ˚C for 16h. After this time, the reaction was concentrated and directly loaded onto silica for purification. The aminobenzothiazole **5.58** desired product was isolated as a 1:1 mixture of inseparable diastereomers (0.33 g, 66%) by MPLC purification (SiO_2, 0 → 30% MeOH/CH_2Cl_2): TLC (MeOH/CH_2Cl_2, 1:9 *v*/*v*): R_f = 0.4; ^{1}H NMR (500 MHz, CDCl3): δ 7.48 (app t, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 9.0 Hz, 2H), 7.12 (app t, *J* = 8.0 Hz, 1H), 7.05 (app t, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.74 (app t, *J* = 5.5 Hz, 1H), 3.93-3.88 (m, 2H), 3.74-3.71 (m, 1H), 3.58-3.55 (m, 1H), 1.41-1.33 (m, H), 1.10-0.98 (m, H); ^{13}C NMR (125 MHz, CDCl3): 168.4, 159.3, 151.4, 130.5, 130.1, 128.6, 128.4, 127.9, 127.7, 126.4, 125.9, 122.2, 121.5, 121.4, 120.6, 119.9, 118.7, 115.6, 115.2, 114.7, 74.7, 74.3, 67.2, 62.5, 62.1, multiple peaks from 59.6-56.9, 49.8, multiple peaks from 38.3-29.3, 20.5; IR (thin film): 3428, 2951, 1645, 1548, 1508, 1360 cm^{-1}.



PIB-BTM 5.59. Aminobenzothiazole 5.58 (0.296 g, 0.25 mmol, 1.0 equiv) was weighed and dissolved in CH_2Cl_2 (1.6 mL) followed by Et_3N (0.1 mL, 0.74 mmol, 3.0 equiv). The resulting solution was cooled in a 0 ˚C ice bath for 10 minutes after which time MsCl (30 μL, 0.37 mmol, 1.5 equiv) was added dropwise. The reaction was allowed to proceed at 0 ˚C for 30 minutes then the ice bath was removed and the reaction warmed to 23 ˚C and continued for 3 h. After this time, MeOH (0.1 mL) was added to quench any remaining MsCl followed by Et_3N (0.1 mL). The reaction was heated to 45 ˚C and proceeded overnight. The next day, the reaction was concentrated *in vacuo* and purified directly by MPLC to provide 0.225 g (76%) of PIB-BTM **5.59**. MPLC purification (SiO_2, 0 → 30% MeOH/CH_2Cl_2): TLC (MeOH/CH_2Cl_2, 1:9 *v*/*v*): R_f =

0.4 ; NMR data is provided for the mixture of diastereomers (1:1 by crude NMR) after purification some separation was achieved, providing a final ratio of 2.5:1 for which the NMR data is reported; $[\alpha]_D^{20}$ = +15.8 (*c* = 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (app d, *J* = 8.5 Hz, 1H), 7.28 (app d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.5, 2H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.16 (dt, *J* = 7.5, 1.0 Hz, 2H), 6.95 (dt, *J* = 7.5, 1.0 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.65 (d, *J* = 7.5 Hz, 2H), 5.60 (dd, *J* = 8.5, 1.5 Hz, 1H), 4.87 (app t, *J* = 8.0 Hz, 1H), 4.67 (app t, *J* = 9.0 Hz, 1H), 4.22 (app t, *J* = 9.0 Hz, 1H), 4.14 (dd, *J* = 7.0, 1.5 Hz, 1H), 3.75 (dd, *J* = 5.5, 3.0 Hz, 2H), 3.66 (app t, *J* = 8.5 Hz, 1H), 3.59 (dd, *J* = 8.0, 6.5 Hz, 2H), 1.39-1.31 (m), 1.13-1.02 (m), 1.08-0.97 (m); ^{13}C NMR (125 MHz, $CDCl_3$): 166.7, 158.9, 137.2, 134.9, 128,2, 128.0, 127.4, 127.2, 127.0, 126.4, 123.8, 123.4, 123.0, 122.8, 122.0, 121.2, 115.5, 115.1, 114.9, 114.5, 108.7, 108.1, 74.7, 74.3, multiple peaks from 59.6-56.9, 52.7, 52.5, 49.8, multiple peaks from 38.3-29.3, 20.5; IR (thin film): 3434, 2961, 1639, 1511, 1469, 1369, 1241 cm^{-1}.



Typical procedure for kinetic resolution of 1-(1-naphthyl)ethanol (5.50). A solution of PIB-BTM **5.59** (4.2 mg, 3.4 μmol, 4mol%) was prepared in heptane (0.6 mL). 1-(1-naphthyl)ethanol (**5.50**) (14.4 mg, 84 μmol, 1.0 equiv) was added followed by PhMe (0.2 mL) and ^{i}Pr$_2$Net (11 μL, 63 μmol, 0.75 equiv). The solution was cooled to 0 ˚C and $(EtCO)_2O$ (8 μL, 63 μmol, 0.75 equiv) was added. The reaction was allowed to proceed for 2.5 h after which it time it was concentrated. A small aliquot was taken for HPLC analysis and separated using known conditions.[2] To the crude reaction mixture, CH_3CN was added and a film formed on the bottom of the vial. The solution was placed on ice for 30 minutes and the CH_3CN was removed by

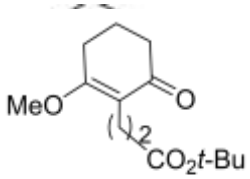
pipette. The remaining **5.59** was washed with more CH_3CN (2 x 1 mL) to provide the clean catalyst. By HPLC analysis (OD-H, 10% ^{i}PrOH, 0.9 mL/min), the conversion was 52%, $ee_{\mathbf{5.50}}$ = 97%, $ee_{\mathbf{5.51}}$ = 88%.

1.) Birman, V. B.; Li, X. *Org. Lett.* **2006**, *8*, 1351-1354.

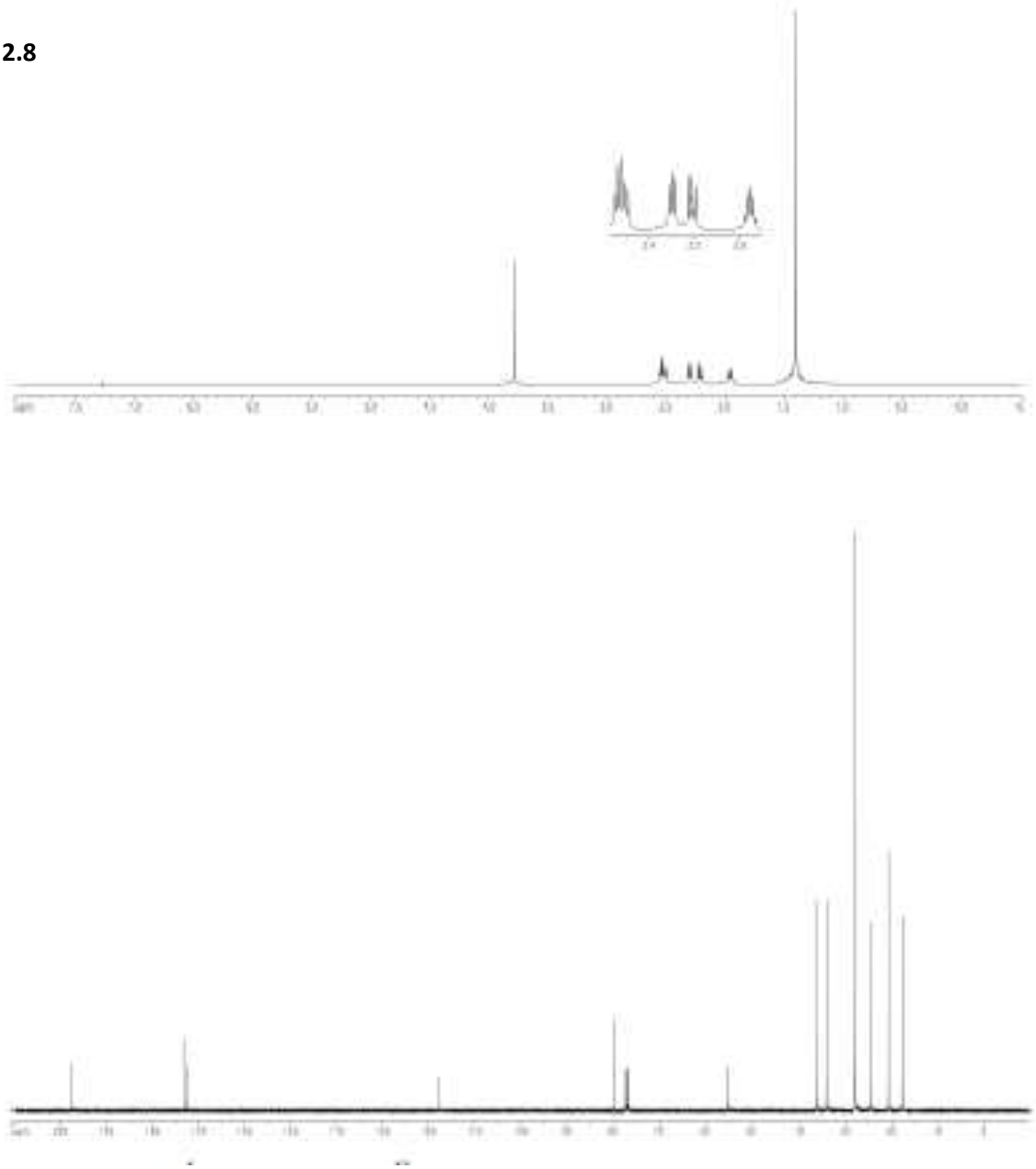
2.) Li, X.; Jiang, H.; Uffman, E. W.; Guo, L.; Zhang, Y.; Yang, X.; Birman, V. B. *J. Org. Chem.* **2012**, *77*, 1722-1737.

APPENDIX B

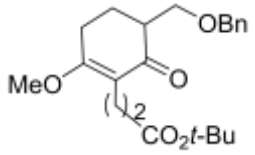
SELECTED SPECTRAL DATA



2.8

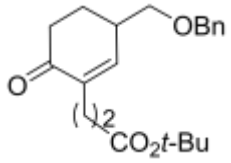


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-keto enol ether **2.8** in $CDCl_3$

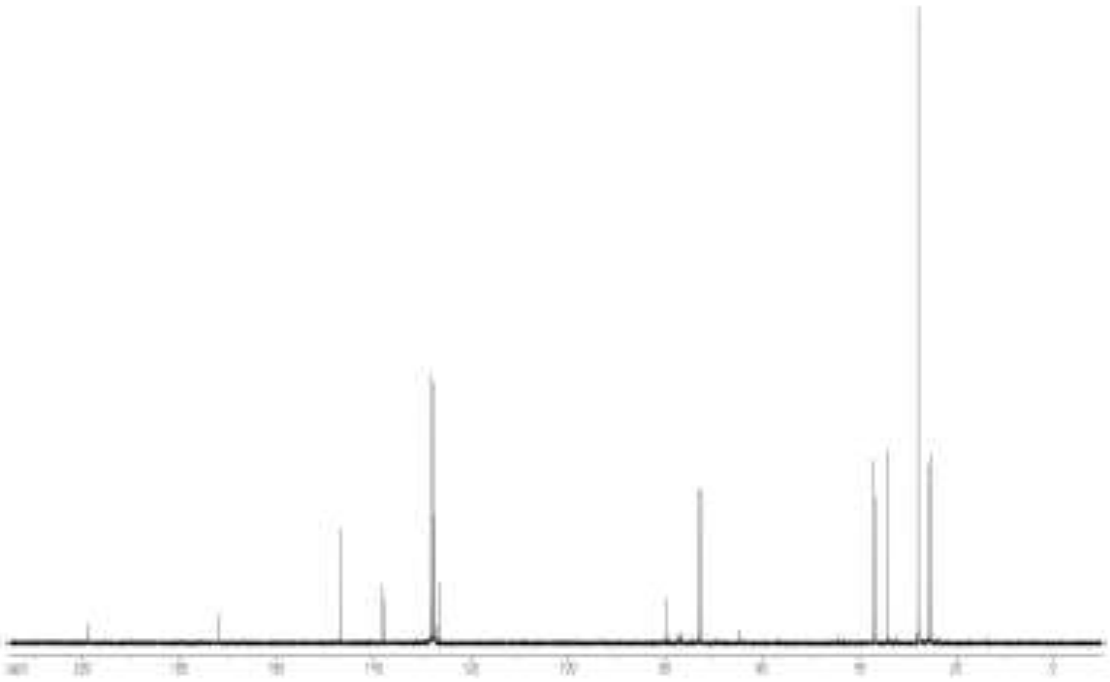


(±)-2.70

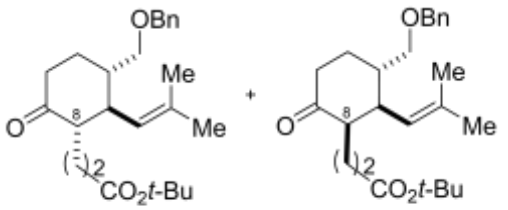
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of benzyl ether (**±**)-**2.70** in $CDCl_3$



(±)-2.7

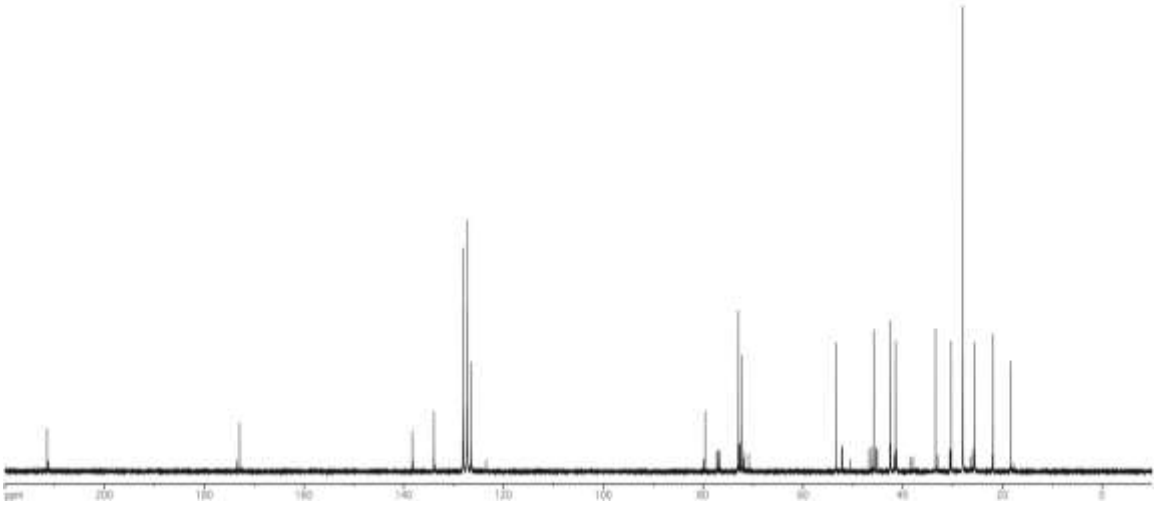


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of cyclohexenone **(±)-2.7** in $CDCl_3$

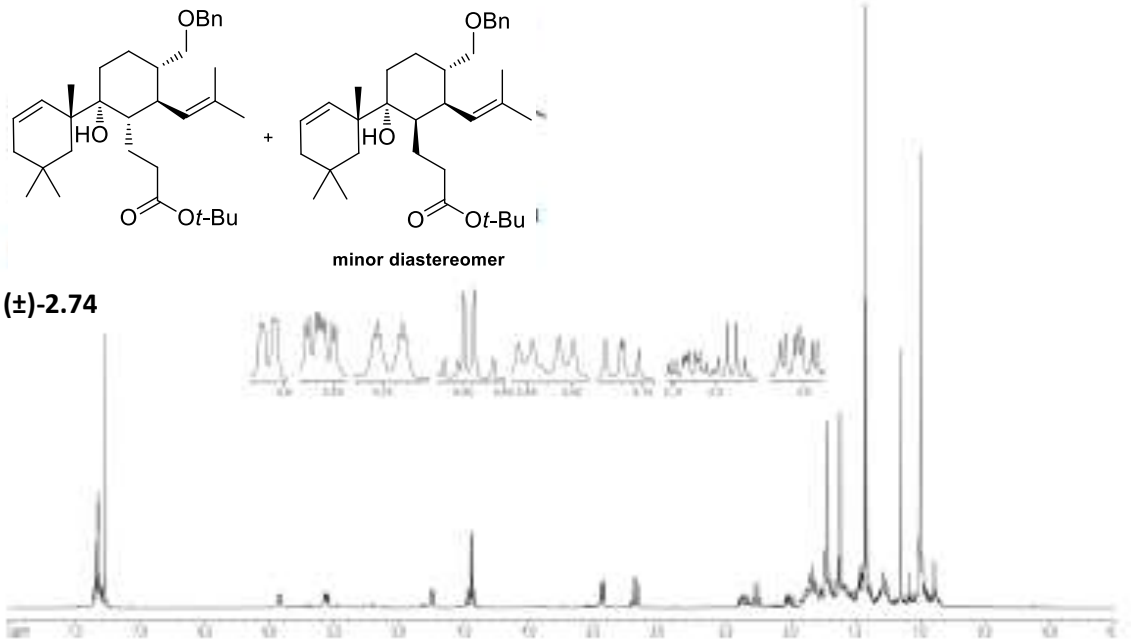


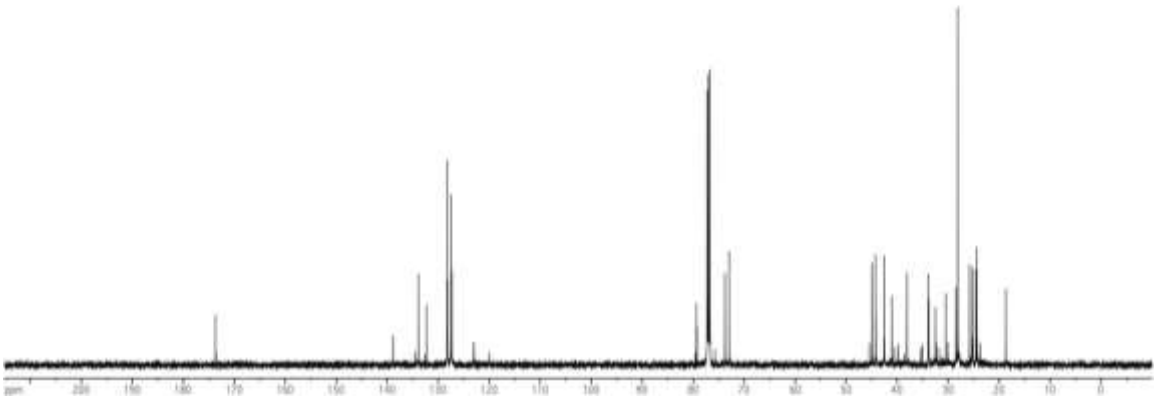
minor diastereomer

(±)-2.6

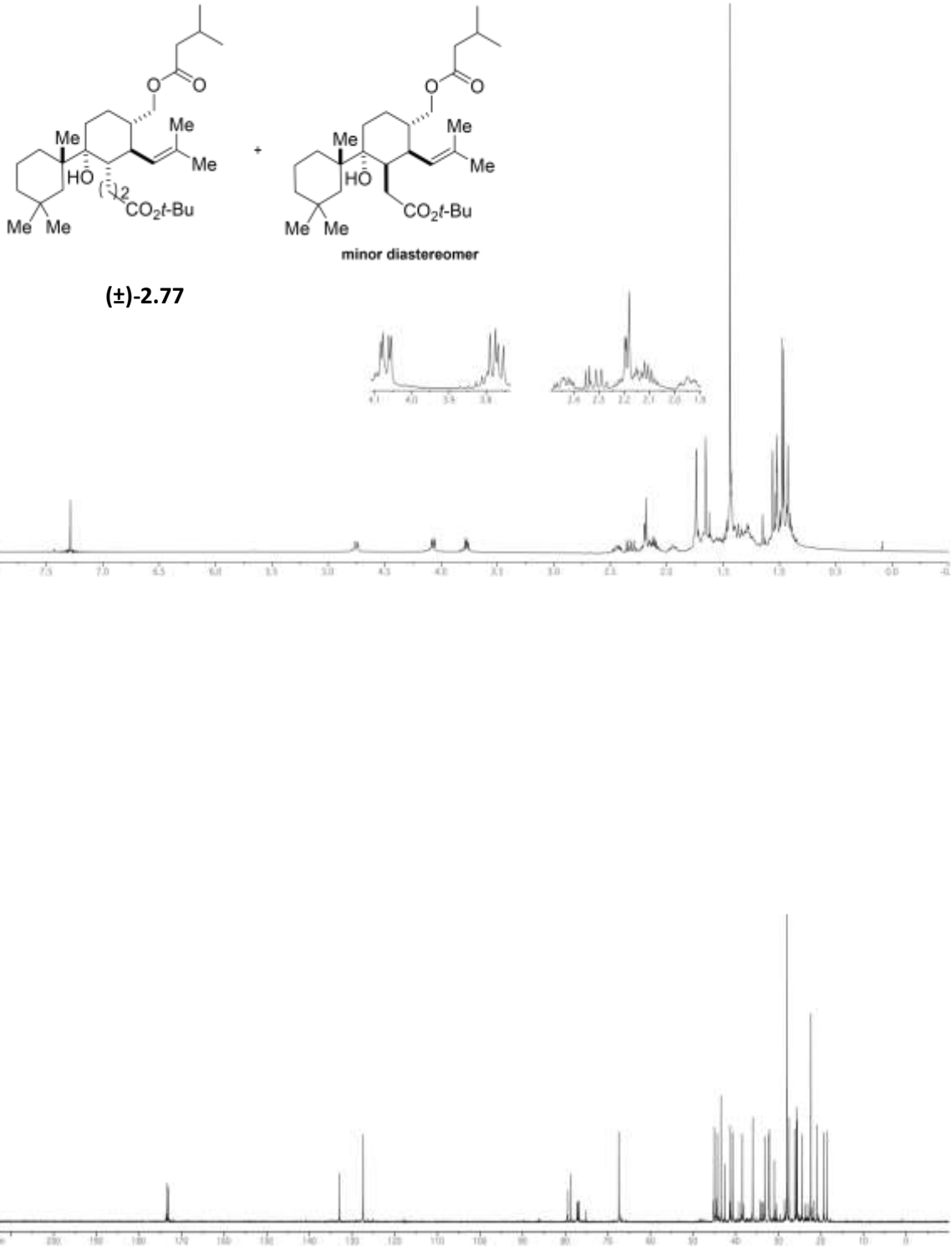


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of tri-substituted cyclohexanone (**±)-2.6** in $CDCl_3$



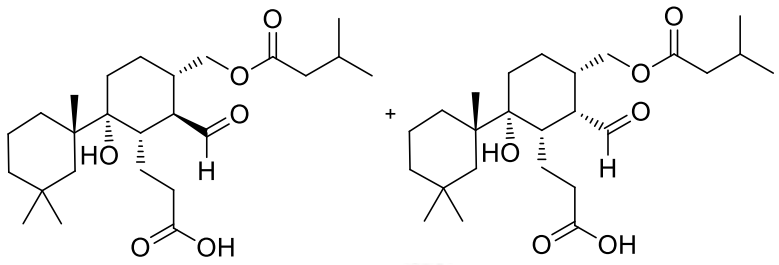


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of homoallylic alcohol (**±)-2.74** in $CDCl_3$

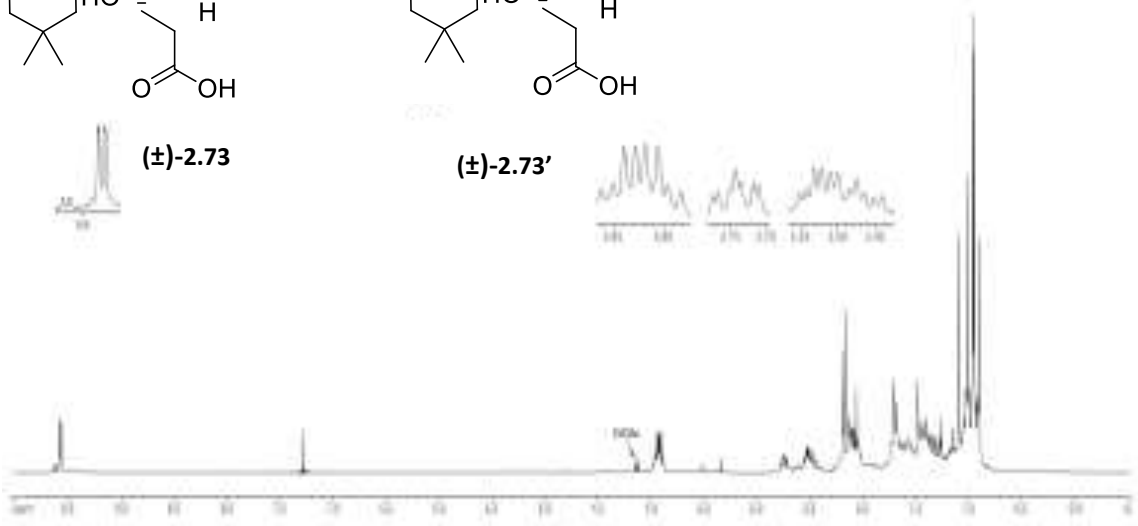


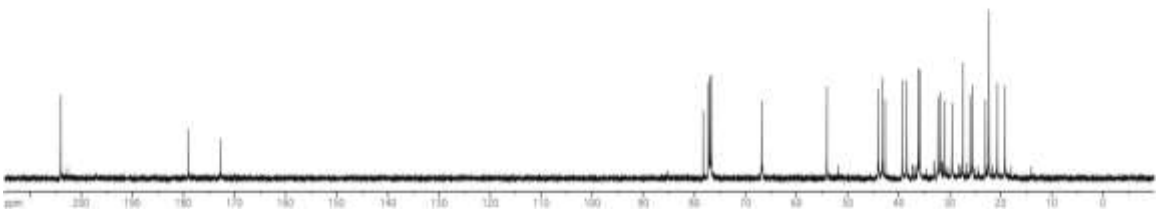
(±)-2.77

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of isovaleric ester (**±)-2.77** in $CDCl_3$

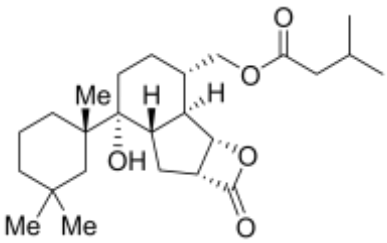


(±)-2.73 **(±)-2.73'**

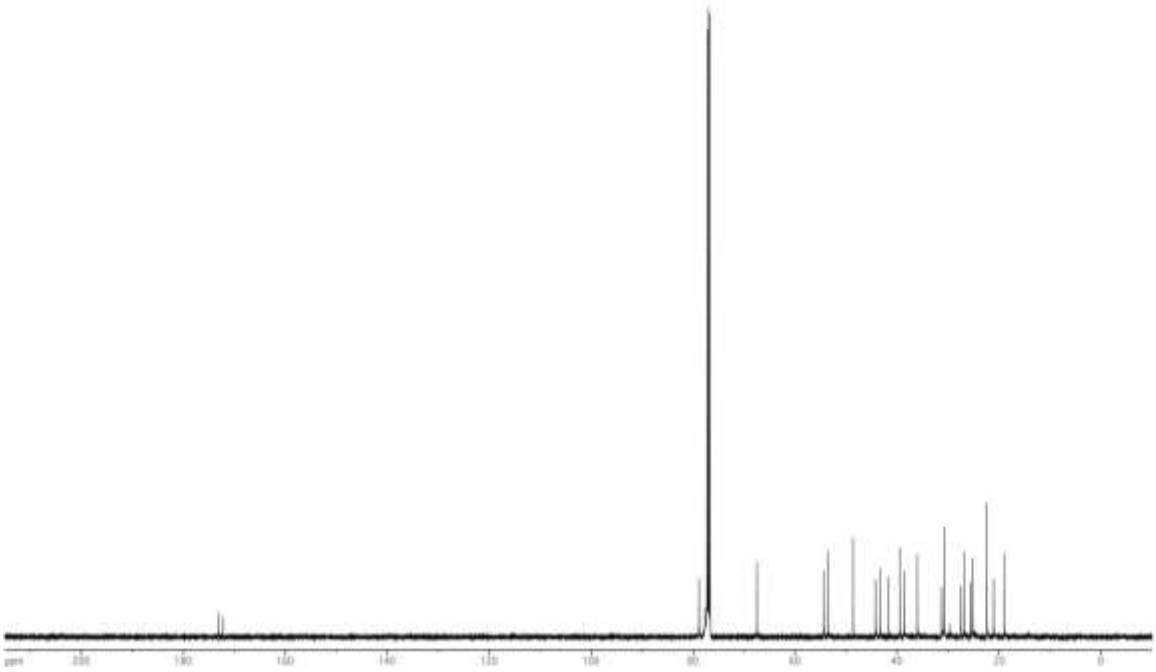




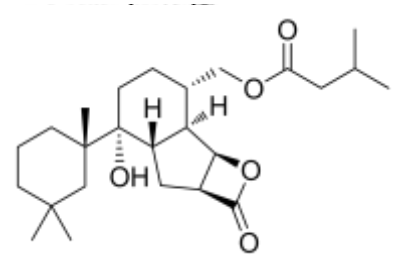
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of aldehyde acid (**±)-2.73** in $CDCl_3$



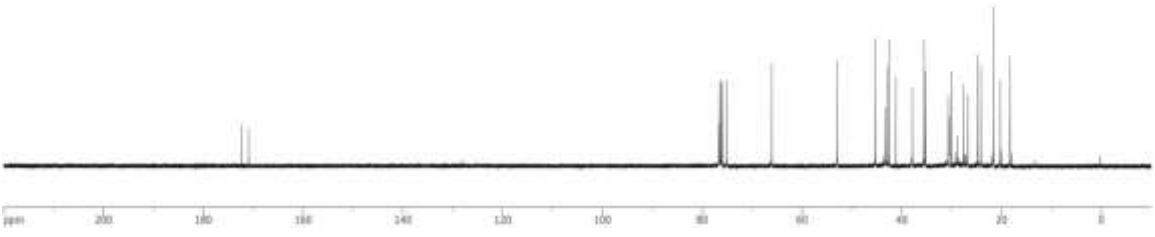
(+)-3.13



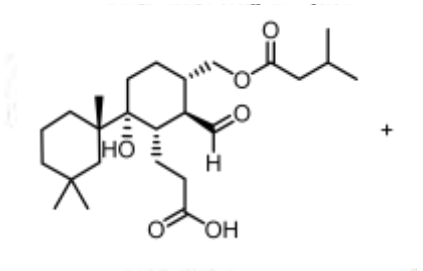
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-lactone (+)-**3.13** in $CDCl_3$



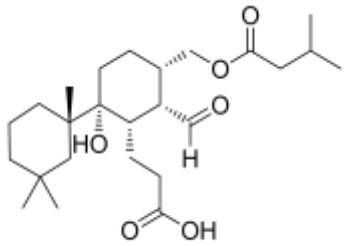
(-)-3.13



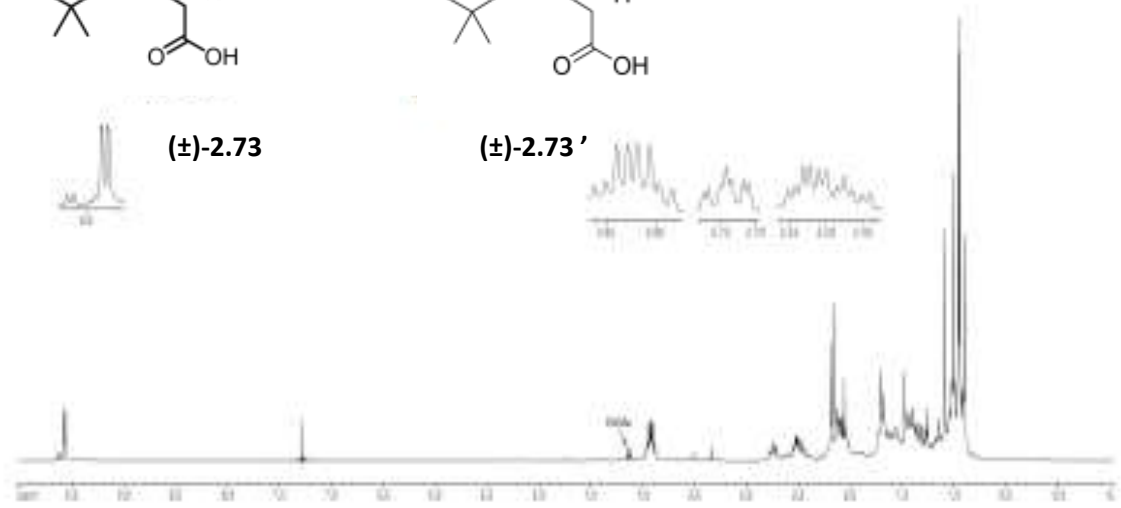
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-lactone (-)-**3.13** in $CDCl_3$

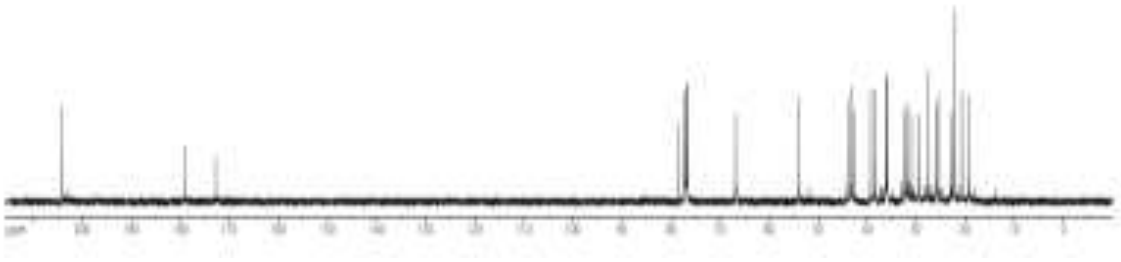


+

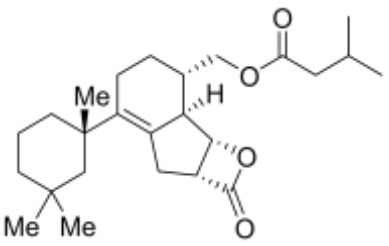


(±)-2.73 **(±)-2.73 ’**

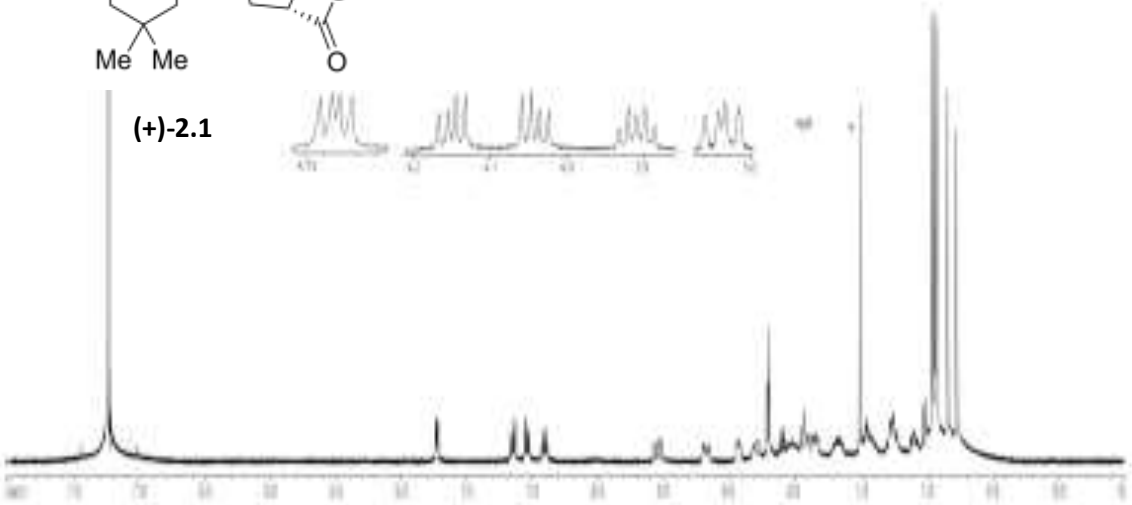


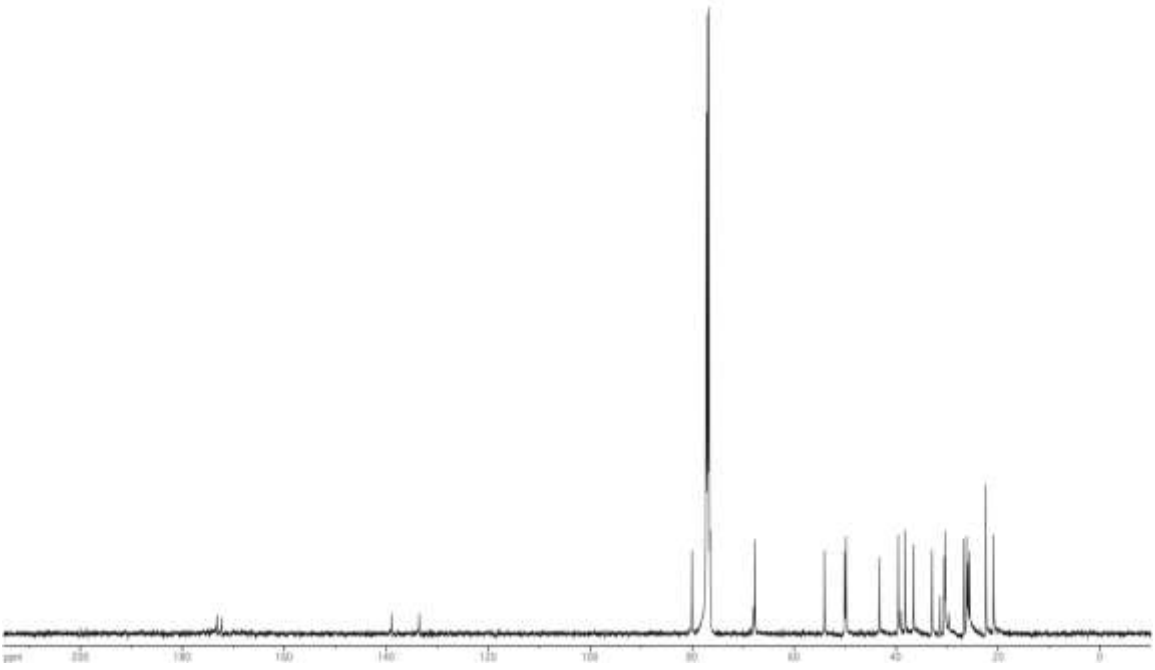


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of epimerized (**±**)-**2.73** in $CDCl_3$

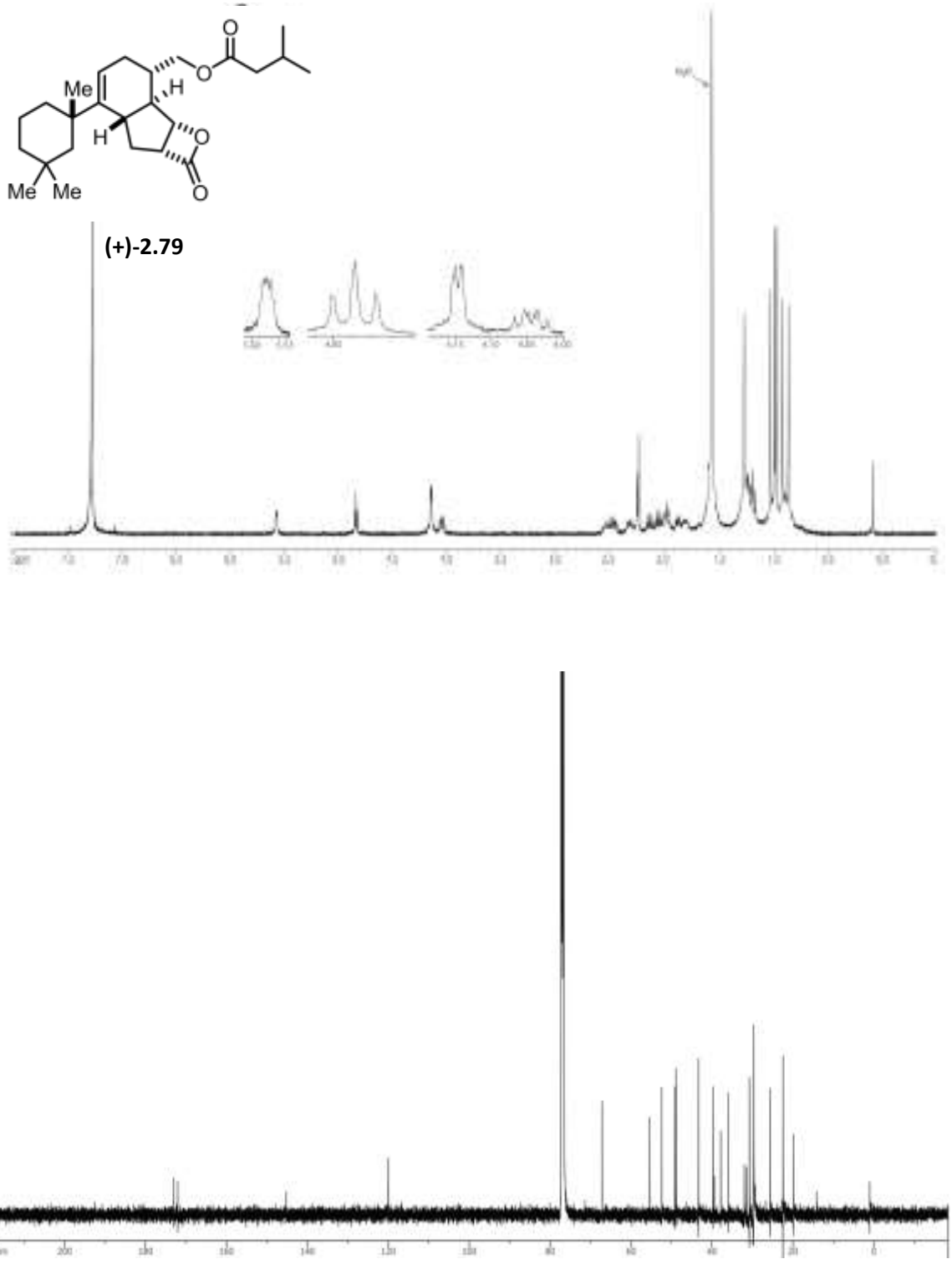


(+)-2.1

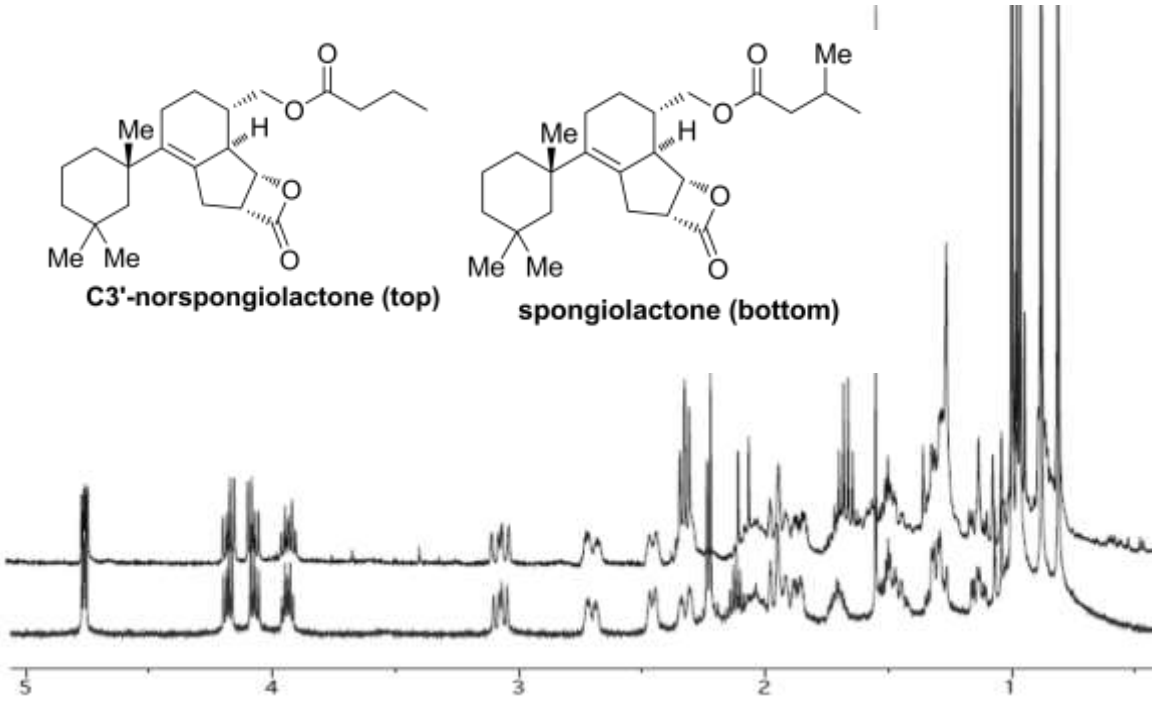




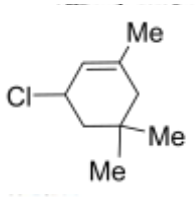
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of spongiolactone (+)-**2.1** in $CDCl_3$



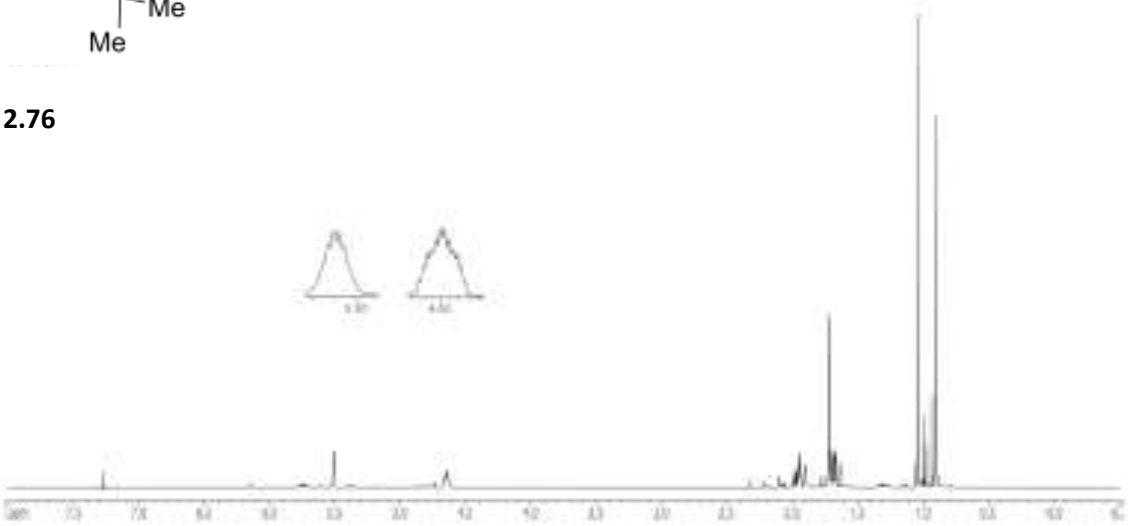
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of regioisomeric spongiolactone (+)-**2.79** in $CDCl_3$

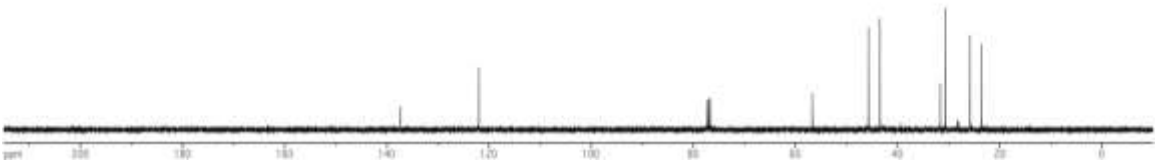


Overlay of ^{1}H NMR (500 MHz, $CDCl_3$) of C3'-norspongiolactone (natural, top) and spongiolactone (synthetic, bottom)

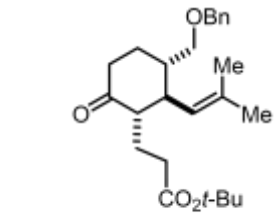


2.76



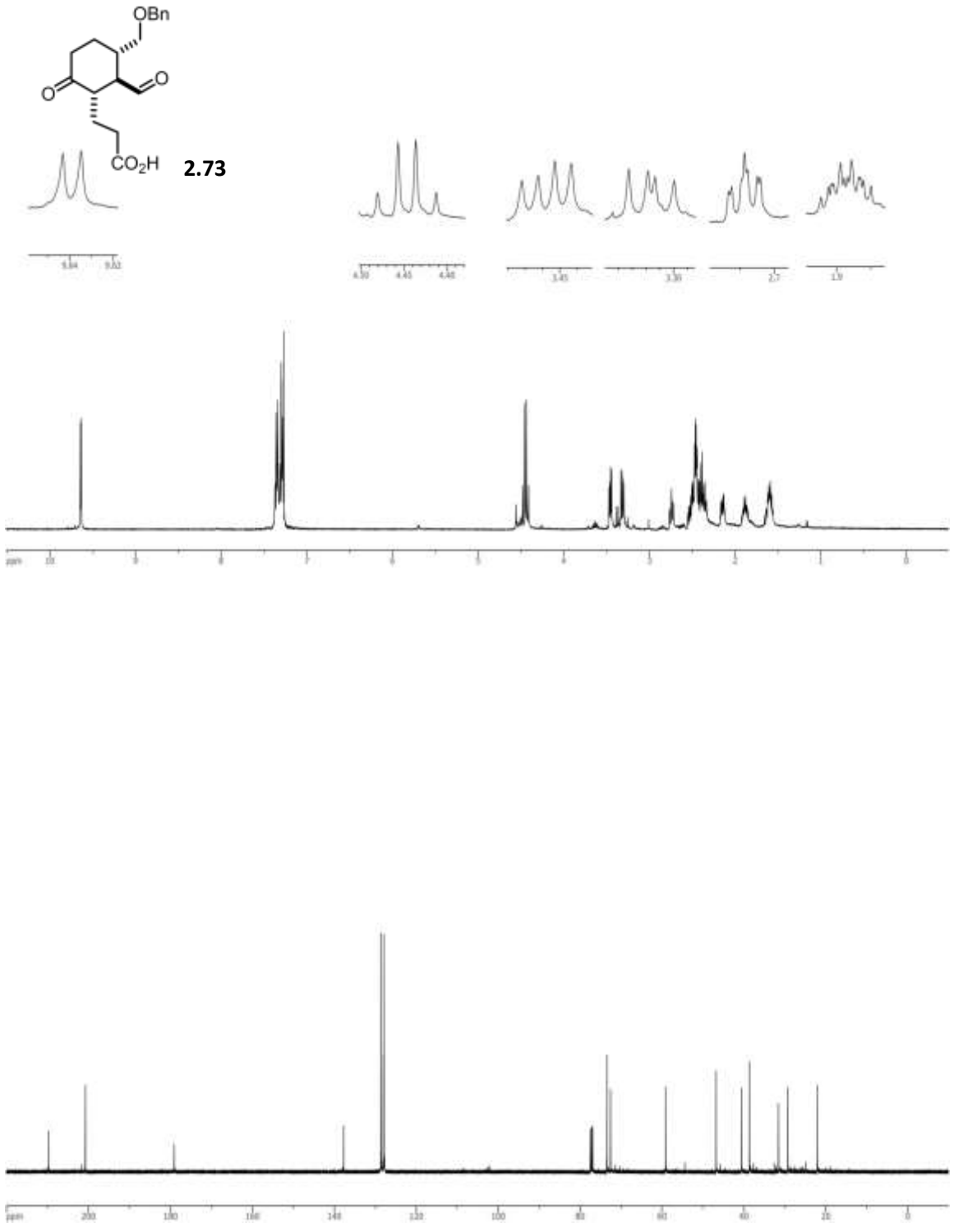


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of allyl chloride **2.76** in $CDCl_3$



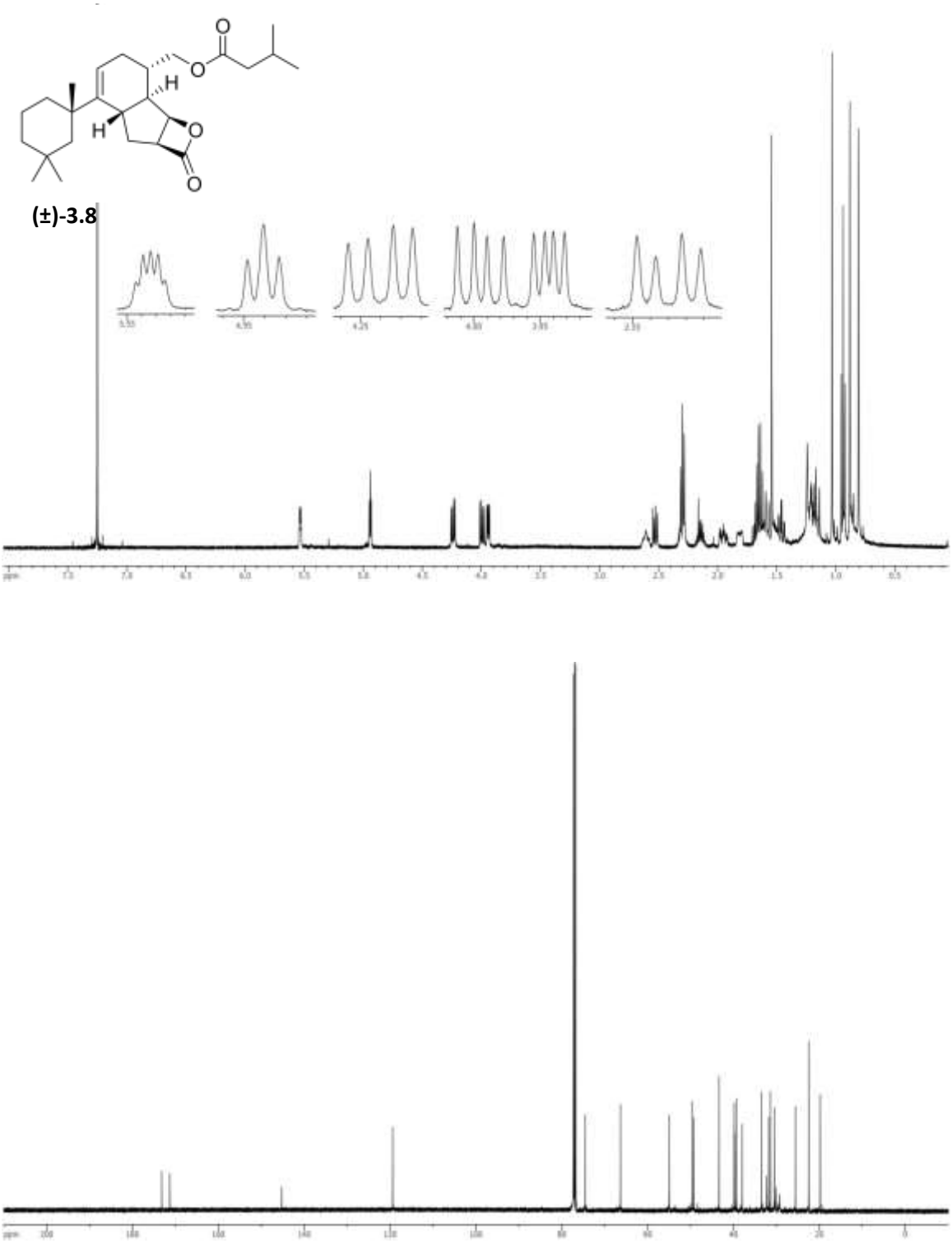
2.77

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of alkene acid **2.77** in $CDCl_3$



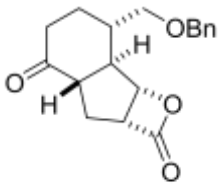
2.73

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of aldehyde acid (±)-**2.73** in $CDCl_3$

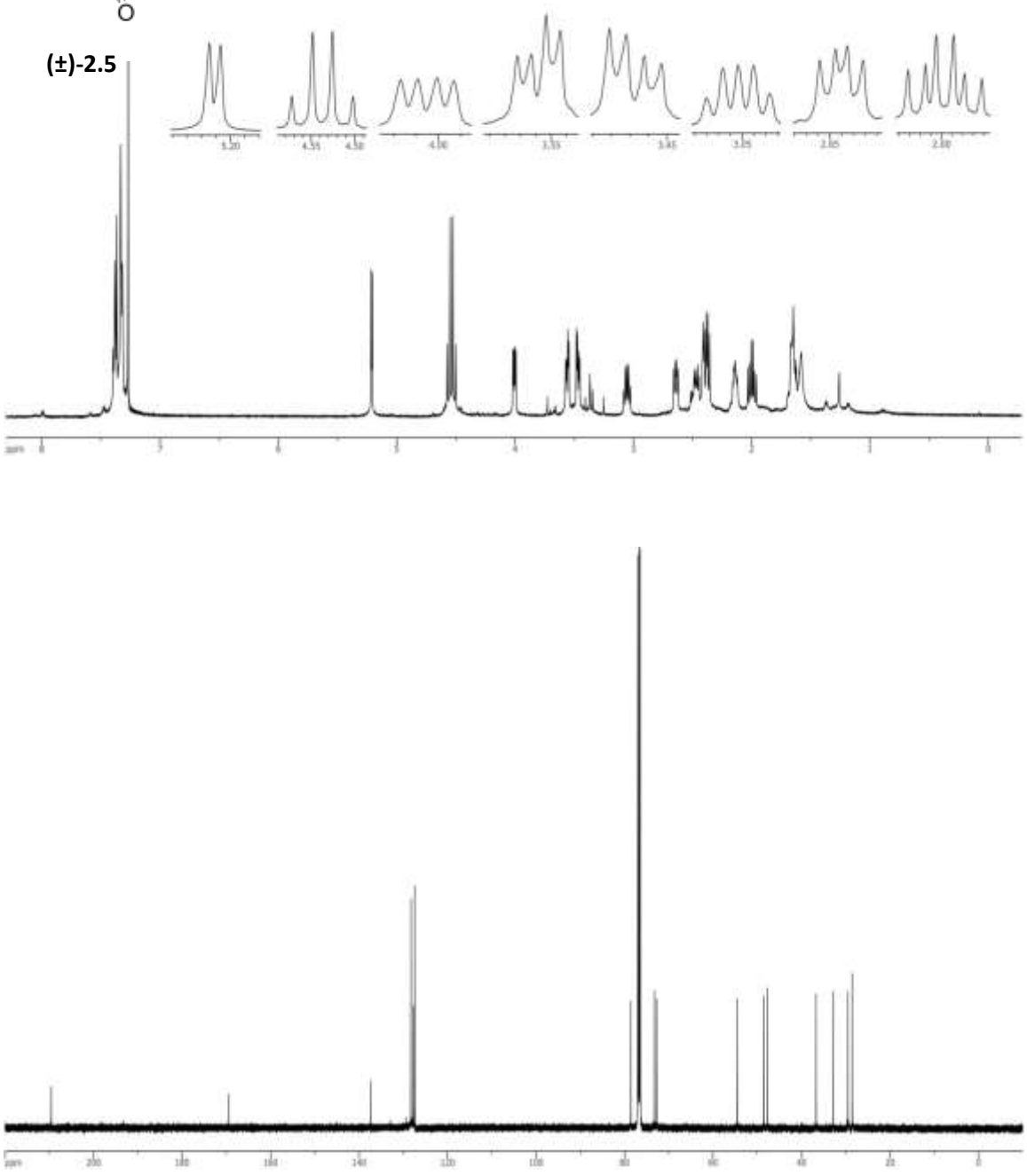


(±)-3.8

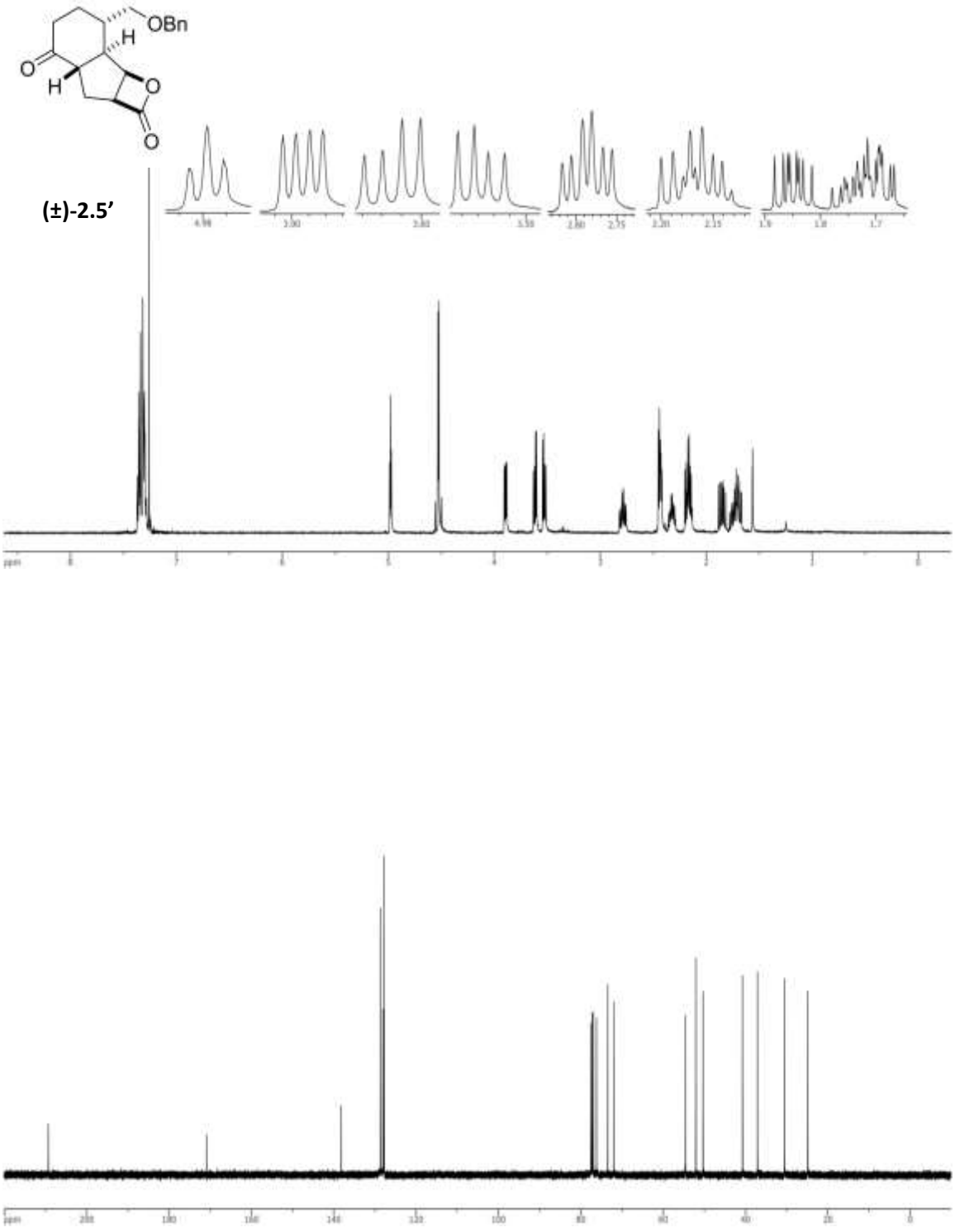
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-lactone (±)-**3.8** in $CDCl_3$



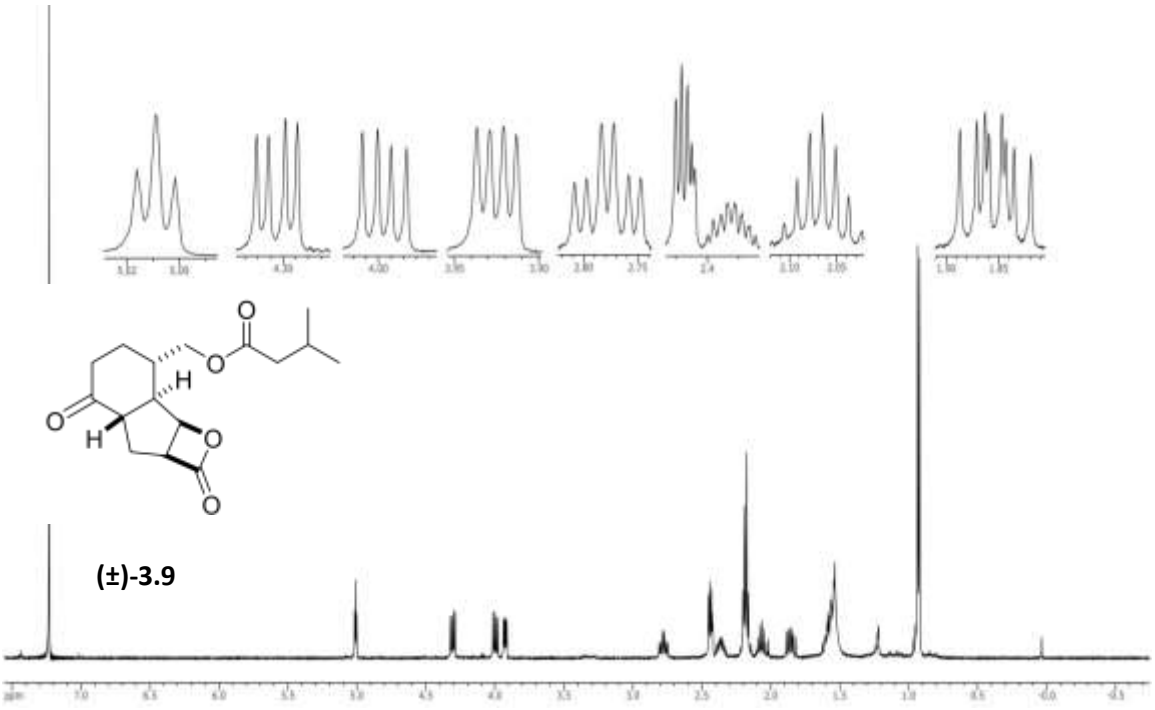
(±)-2.5

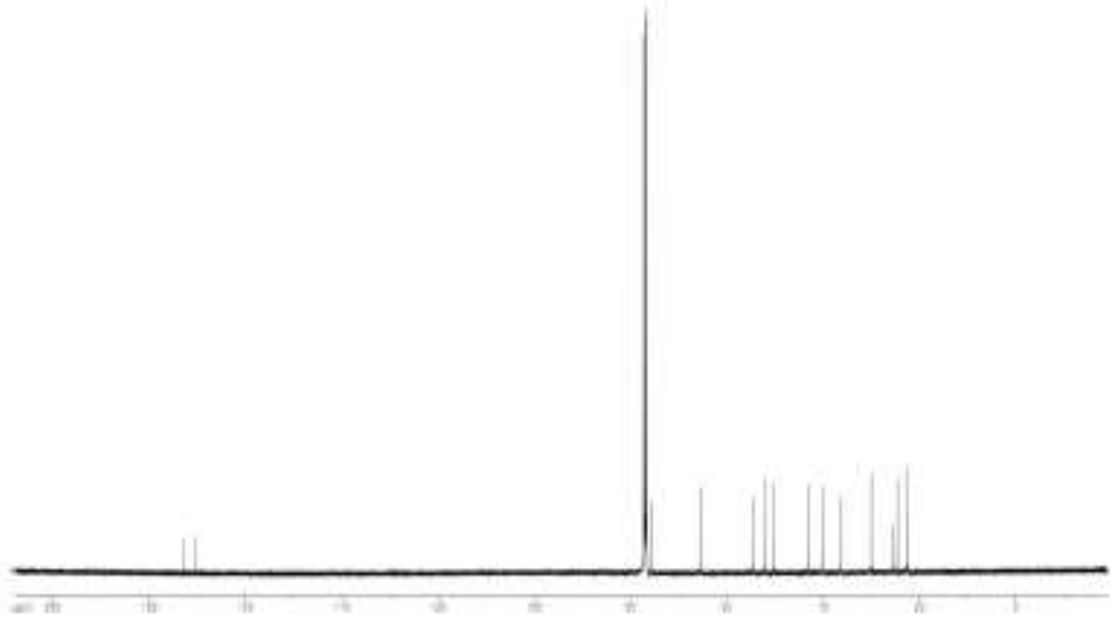


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-lactone (±)-**2.5** in $CDCl_3$

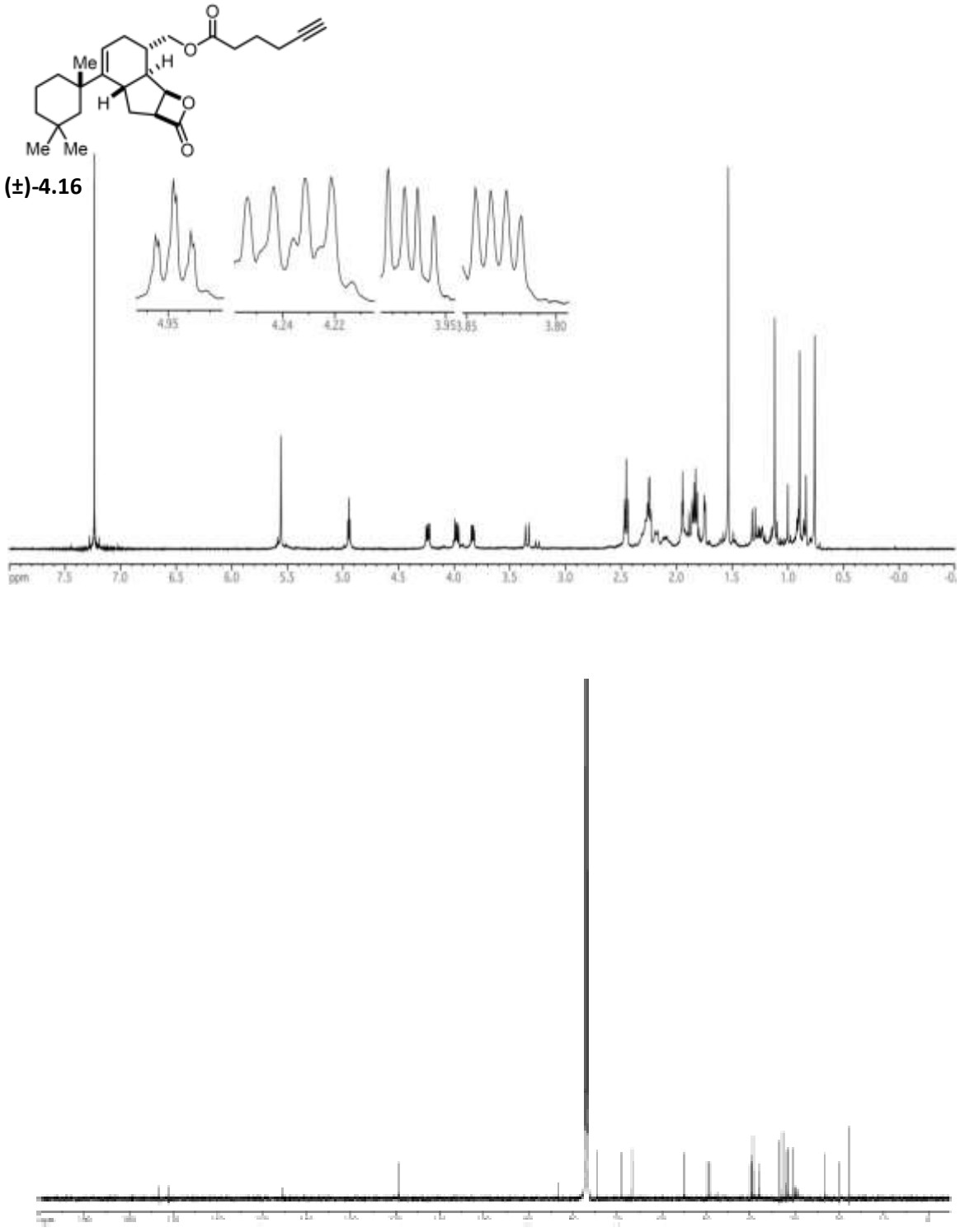


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-lactone (±)-**2.5’** in $CDCl_3$

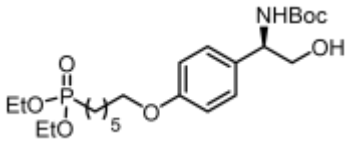




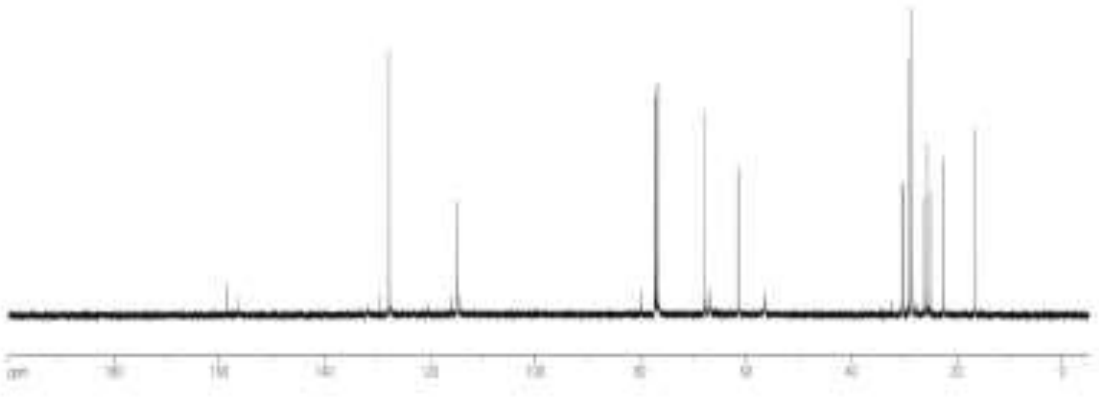
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of β-lactone (±)-**3.9** in $CDCl_3$



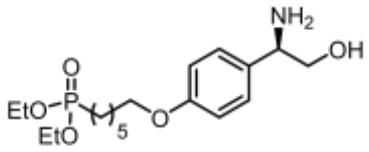
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of alkyne probe (±)-**4.16** in $CDCl_3$



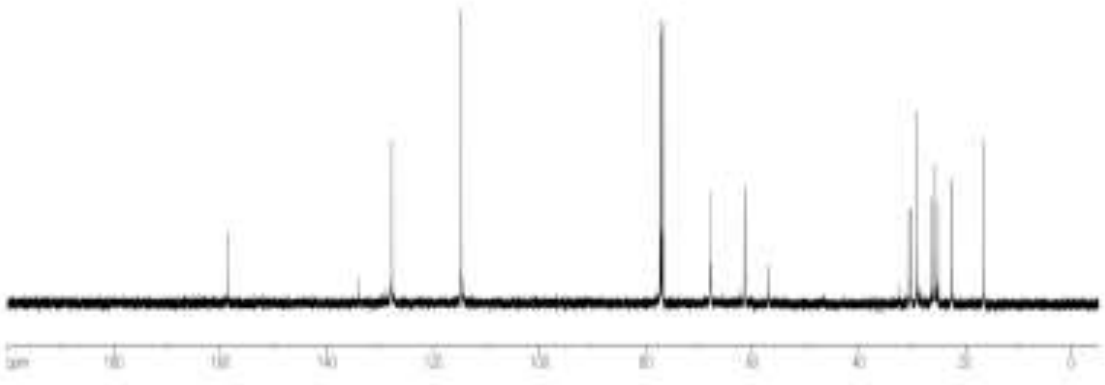
A4



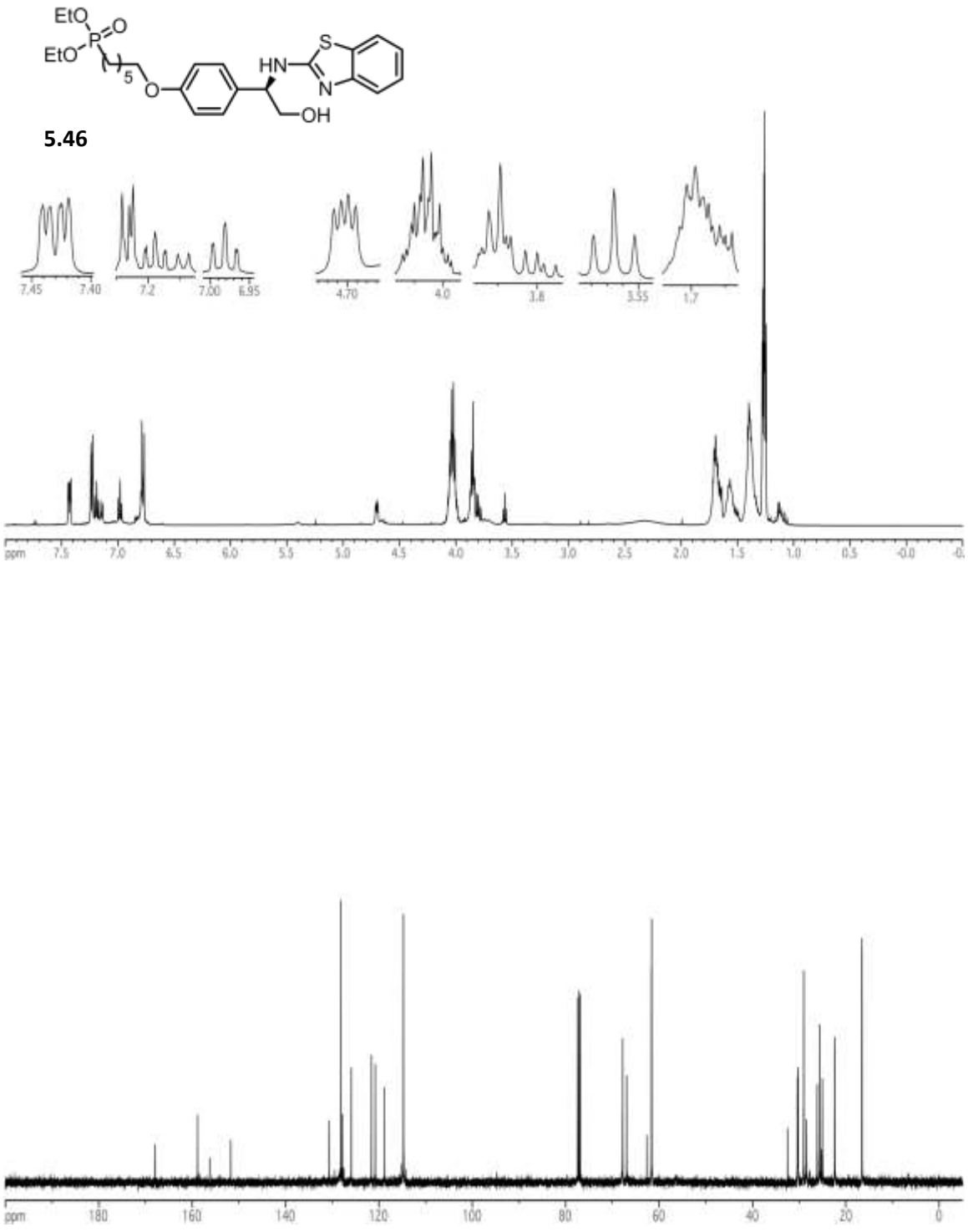
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of **A4** in $CDCl_3$



5.45

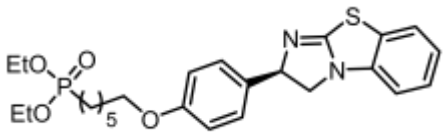


^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of **5.45** in $CDCl_3$

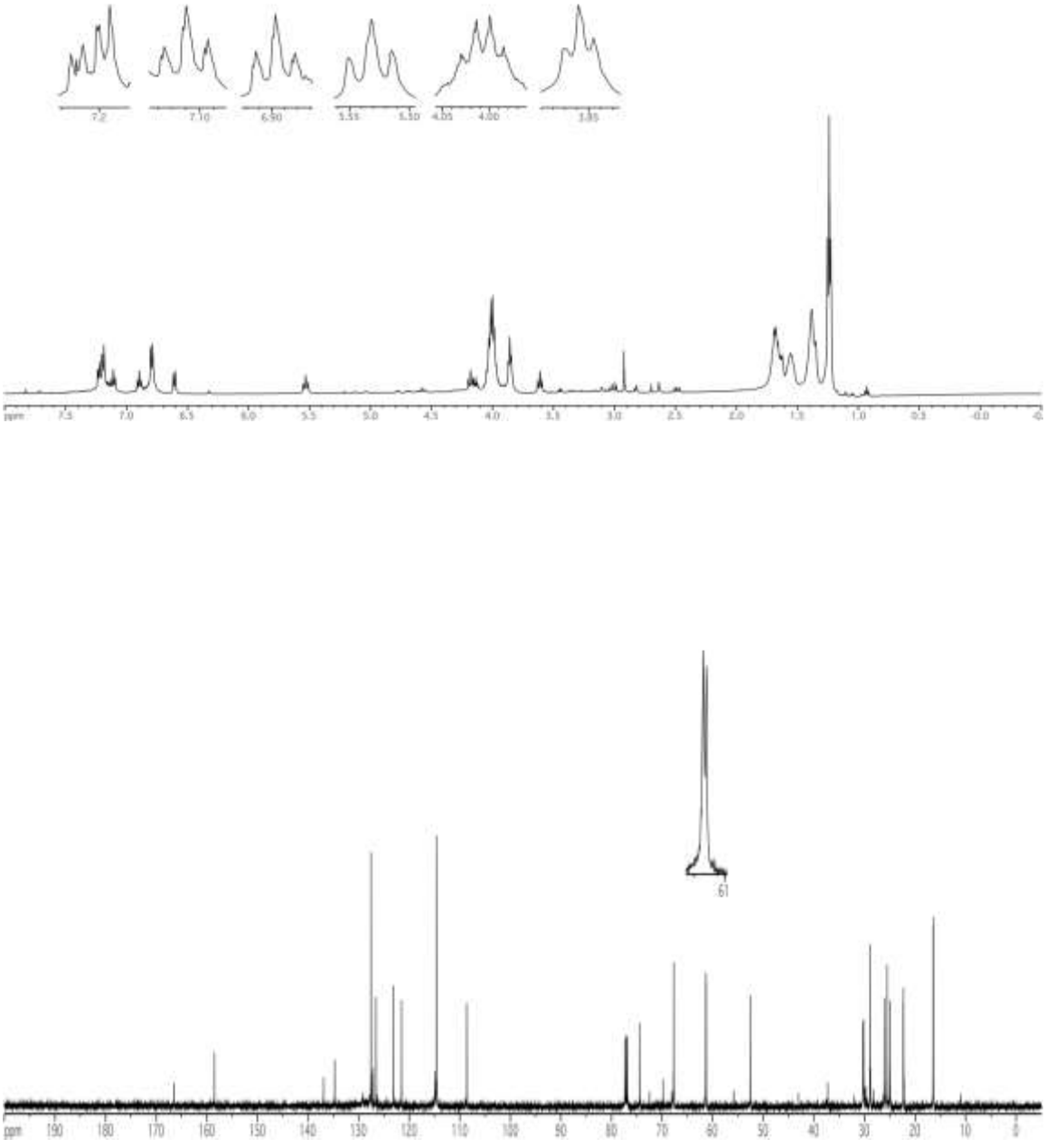


5.46

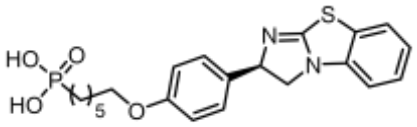
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of **5.46** in $CDCl_3$



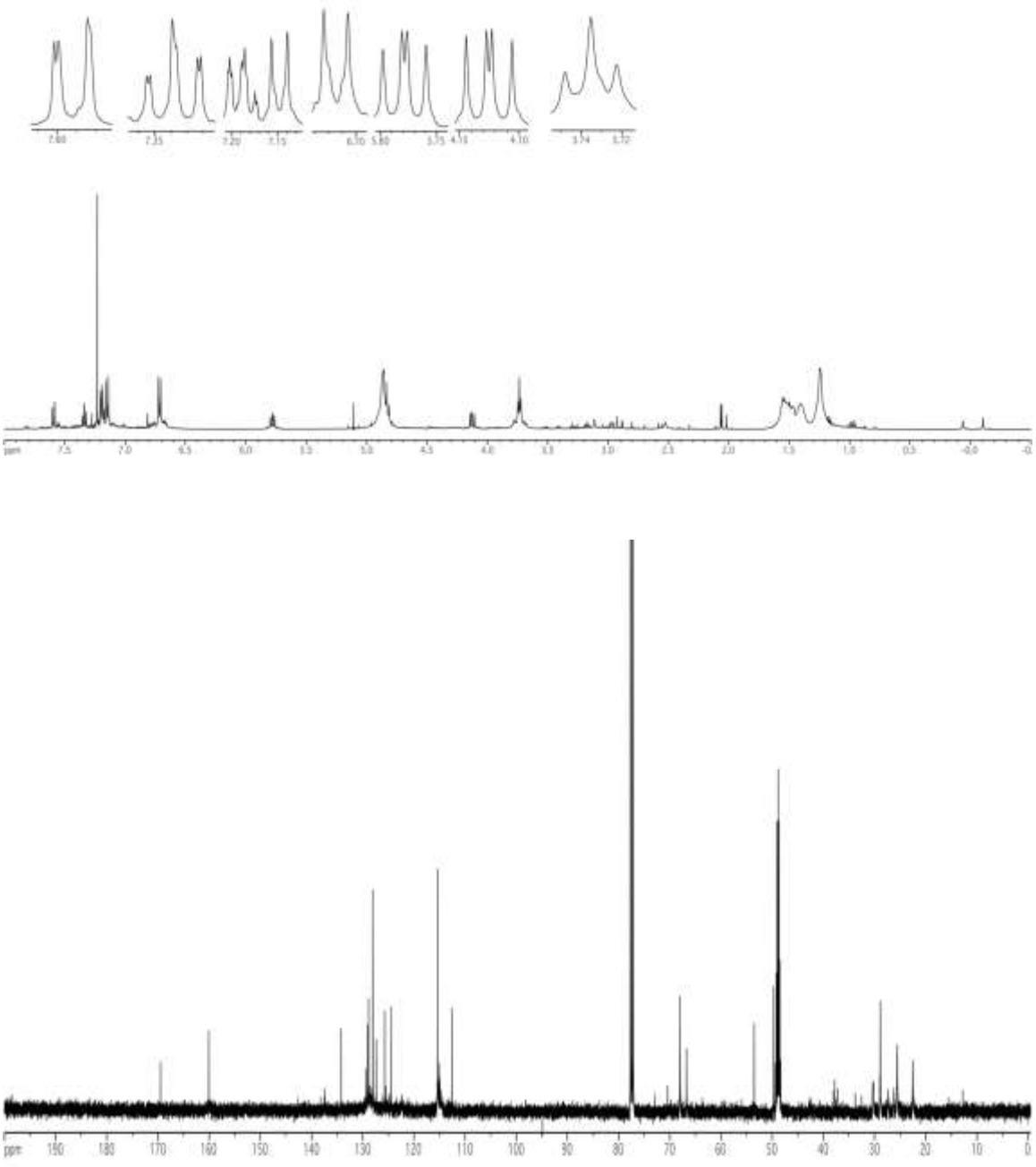
5.47



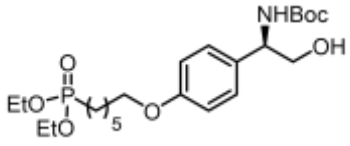
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of **5.47** in $CDCl_3$



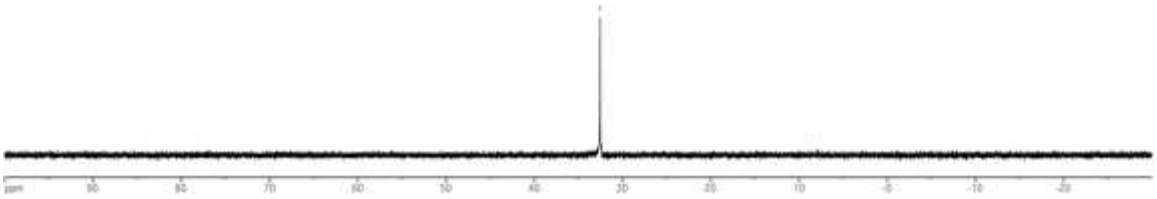
5.48



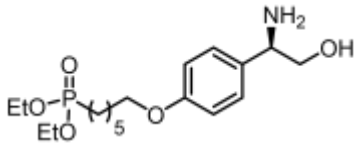
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of **5.48** in $CDCl_3$ and CD_3OD



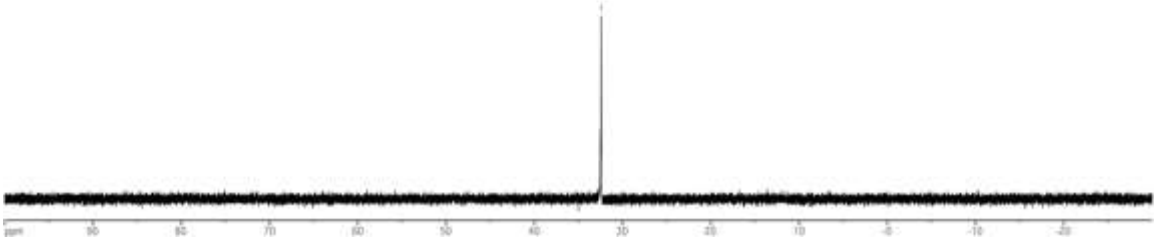
A1



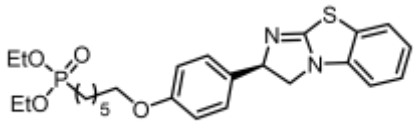
^{31}P NMR (202 MHz) of **A4** in CD_3OD



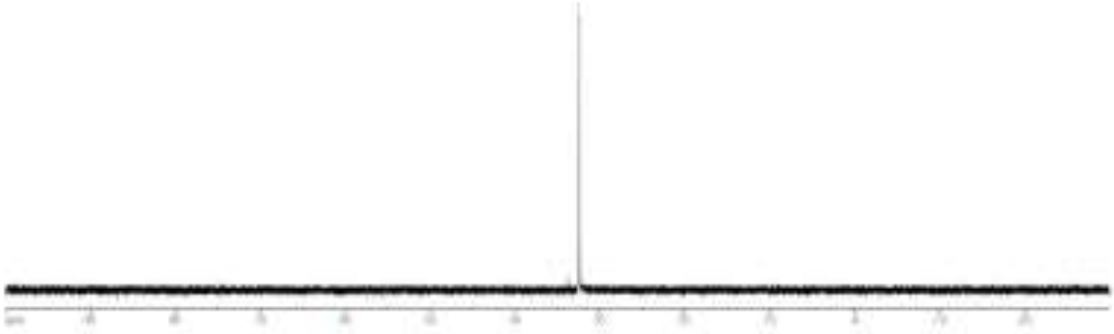
5.45



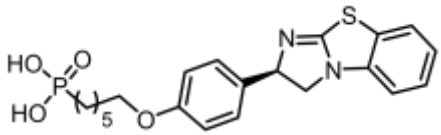
^{31}P NMR (202 MHz) of **5.45** in CD_3OD



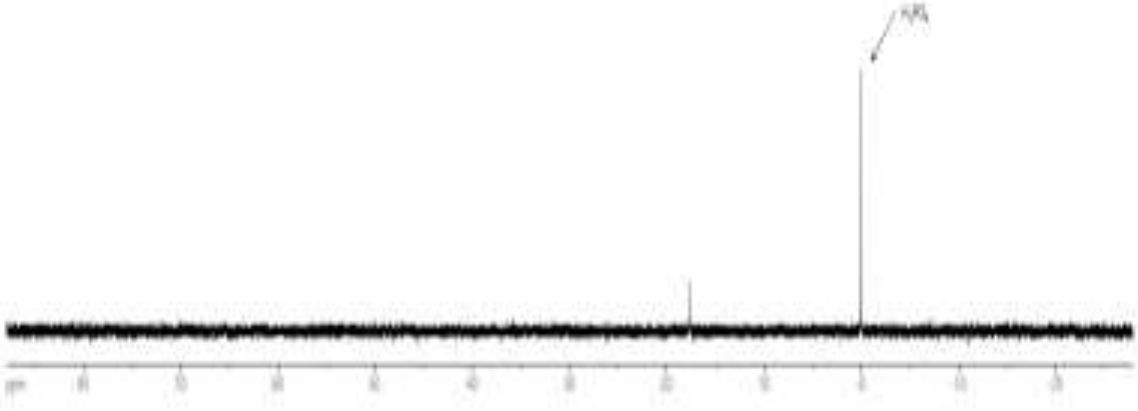
A1



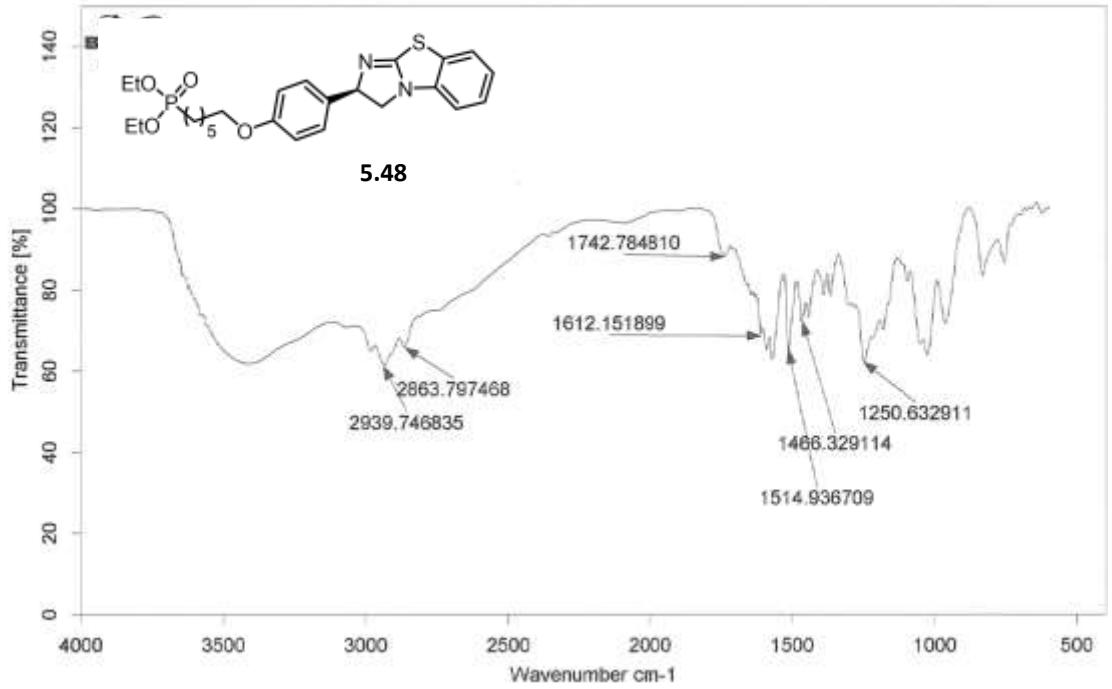
^{31}P NMR (202 MHz) of **A4** in CD_3OD



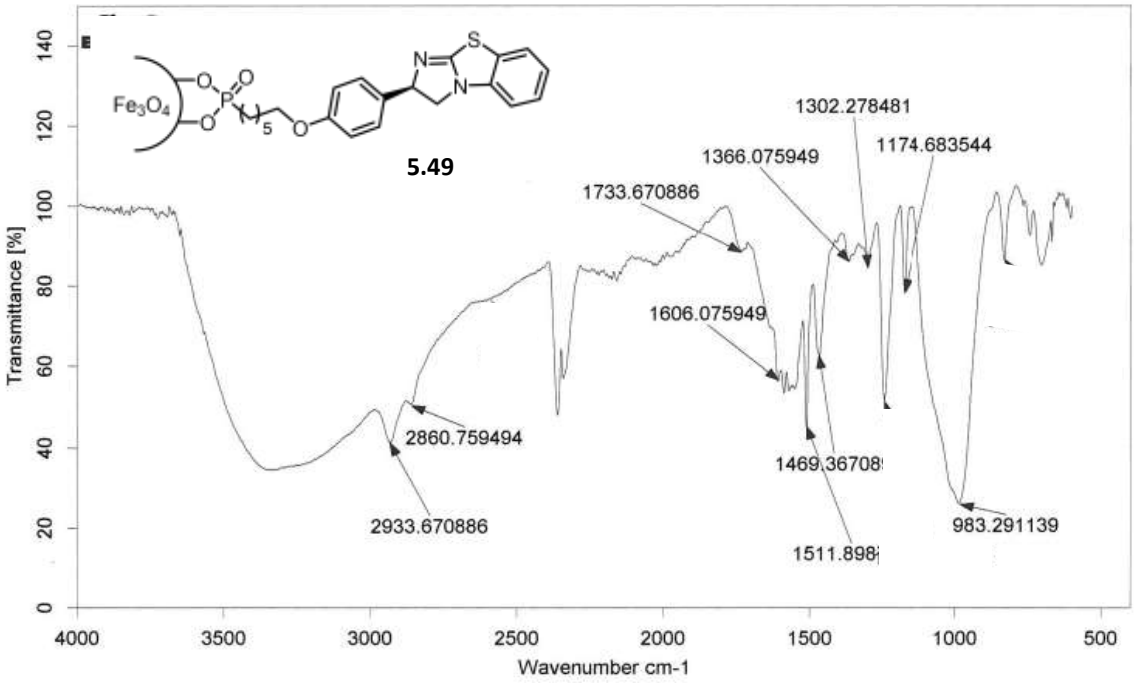
5.45



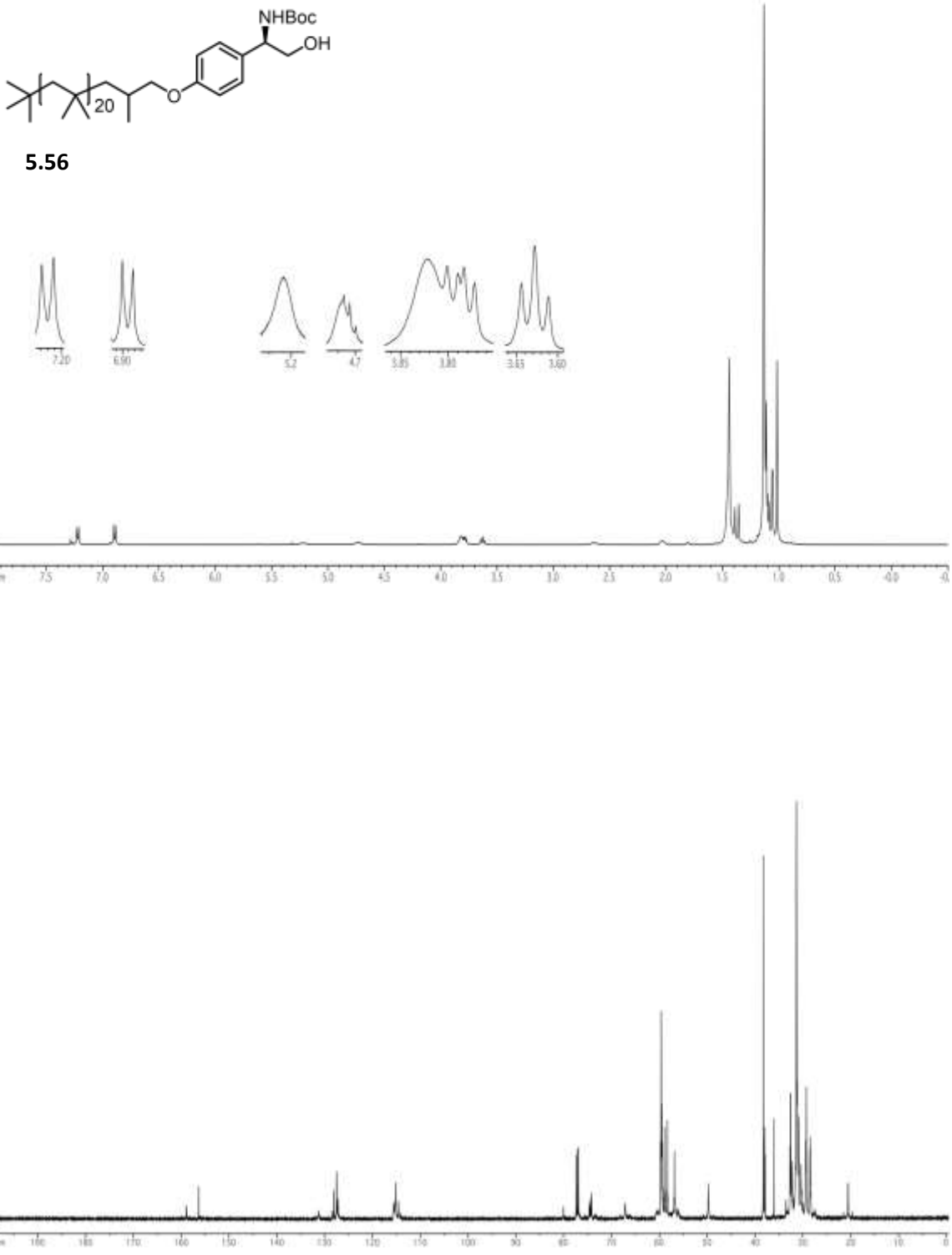
^{31}P NMR (202 MHz) of **5.45** in CD_3OD



IR spectrum of **5.48**

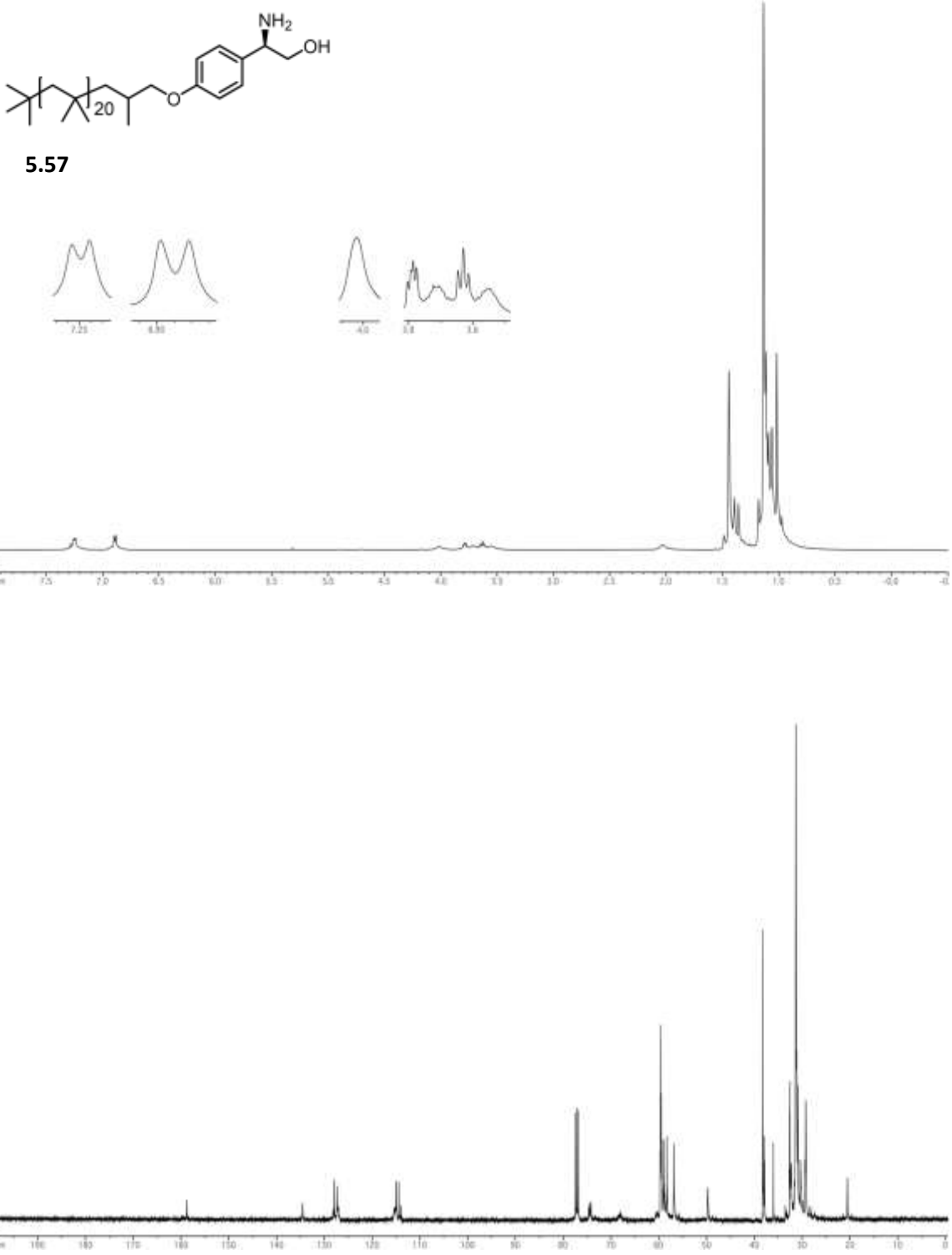


IR spectrum of **5.49**



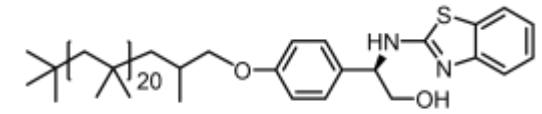
5.56

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) of **5.56** in $CDCl_3$

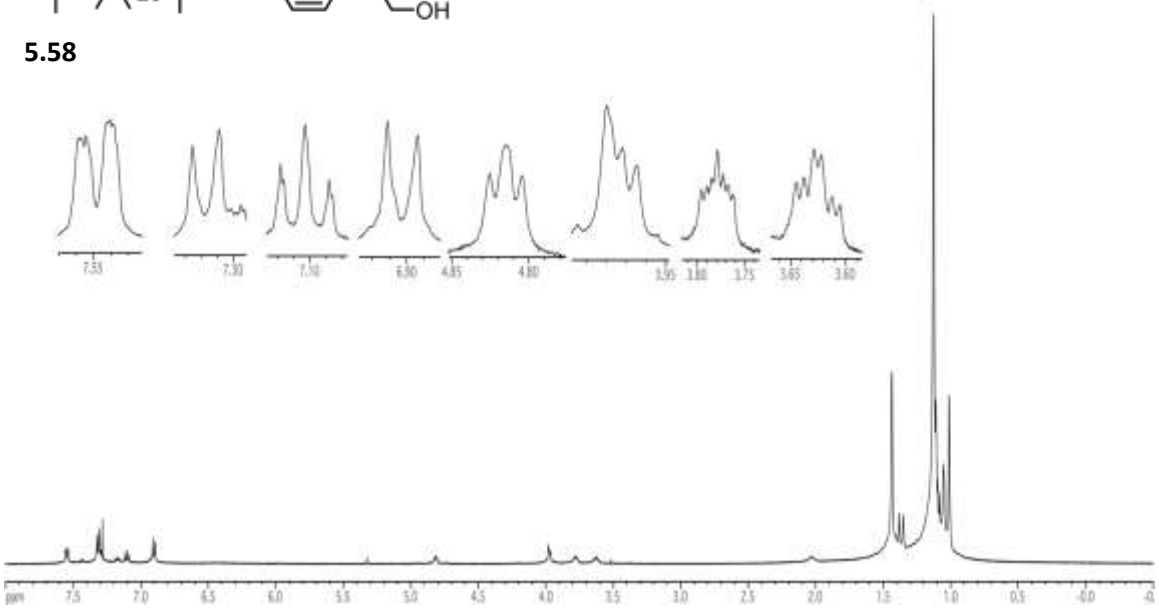


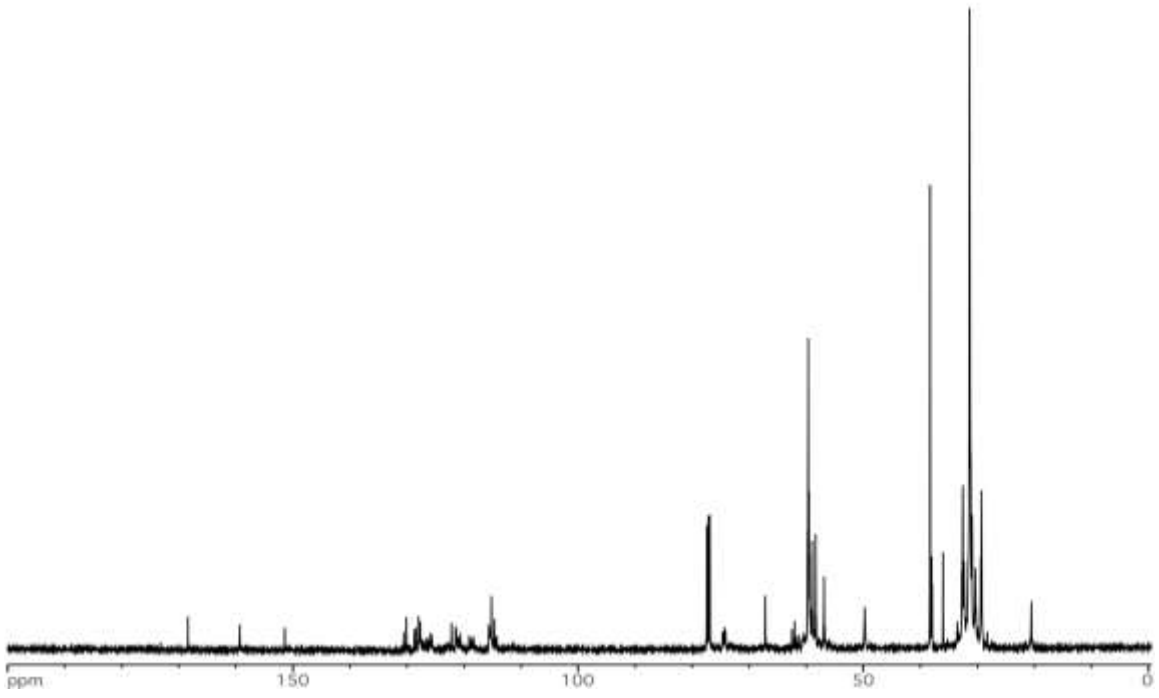
5.57

(500 MHz) and ^{13}C NMR (125 MHz) of **5.57** in $CDCl_3$

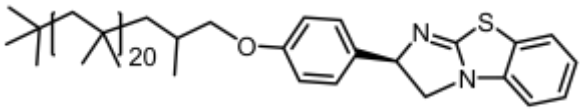


5.58

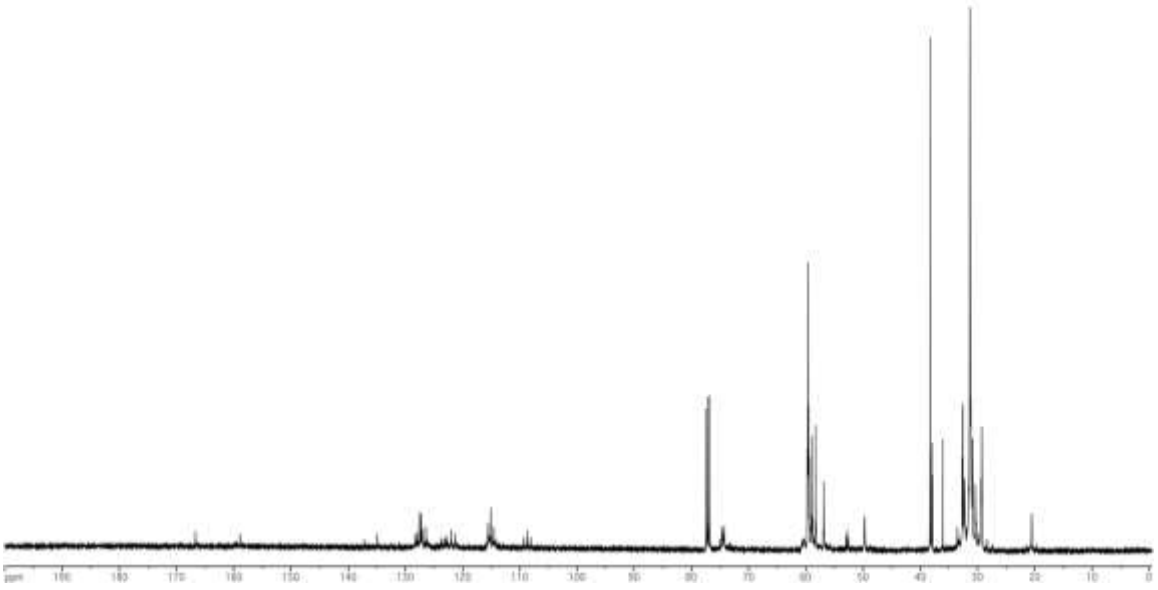




(500 MHz) and ^{13}C NMR (125 MHz) of **5.58** in $CDCl_3$



5.59



(500 MHz) and ^{13}C NMR (125 MHz) of 5.5**9** in $CDCl_3$

APPENDIX C

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